ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH

1959

NATIONAL INSTITUTE OF DENTAL RESEARCH
DIVISION OF BIOLOGICS STANDARDS
CLINICAL CENTER

NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Library
National Institutes of Health
Bethesda, Maryland 20014

RK
80
U563
1959

Report of Program Activities

U.S. NATIONAL INSTITUTE OF DENTAL RESEARCH
Annual Narrative and Project Reports
Calendar Year 1959

CONTENTS

Narrative Reports — Page

Intramural 1-16
Extramural 17-22
Administration 23-24

Project Reports

OFFICE OF ASSOCIATE DIRECTOR

NIDR No.	Investigator(s)	Project Title
1	S. J. Kreshover	Prenatal Influences on Fetal Development.

LABORATORY OF HISTOLOGY AND PATHOLOGY

NIDR No.	Investigator	Project Title
2	D. B. Scott & M. U. Nylen	Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.
3	M. U. Nylen & D. B. Scott	Studies of the Structure of Developing Calcified Tissues by Electron Microscopy and X-ray Microscopy.
4	D. B. Scott & M. U. Nylen	Secondary Collaborative Projects and Training Activities.
5	A. E. W. Miles & D. B. Scott	Age Estimation on the Basis of Histologic Examination of Teeth.
6.	S. Takuma	Electron Microscopic Studies of the Ultrastructure of Developing and Mature Bone and Dentin.
7	P. H. Keyes	Experimental Animal Caries.
8	M. S. Burstone	Diagnostic Oral and Surgical Pathology Utilizing Histochemical Techniques with Emphasis Upon Periodontal Disease and Neoplasia.
9	M. S. Burstone	Development and Application of Histochemical Methods in the Study of Hard and Soft Tissues.
10	H. M. Fullmer	Histochemical Studies of Connective Tissues.

NATIONAL INSTITUTE OF DENTAL RESEARCH
Annual Narrative and Project Reports
Calendar Year 1959

CONTENTS

LABORATORY OF MICROBIOLOGY

NIDR No.	Investigator(s)	Project Title
11	R. J. Fitzgerald & H. V. Jordan	The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease.
12	E. G. Hampp & S. E. Mergenhagen	Studies on Experimentally Induced Fuso-spirochetal Infection in Rabbits.
13	A. Howell, Jr. & L. Pine	A Study of Oral Filamentous Organisms and Related Bacteria.
14	A. Howell, Jr. & A. A. Rizzo	The Relationship of Pathogenic Fungi to Diseases of the Oral Cavity. I. Incidence and Identity.
15	H. V. Jordan & R. J. Fitzgerald	Inhibitors and Experimental Dental Caries.
16	S. E. Mergenhagen & E. G. Hampp	Endotoxins from Oral Bacteria.
17	T. A. Nevin	Studies of Interactions Between Oral Organisms.
18	T. A. Nevin	Bacteremias Following Exodontia and Periodontal Treatment.
19	R. R. Omata	Studies on the Physiology of Oral Fusobacteria and on Proteolytic Enzymes of Microbial Origin.
20	A. A. Rizzo	Methods of Using the Hamster Cheek Pouch for Periodontal Research.
21	A. A. Rizzo	A Comparison of the Pathology of Chronic Magnesium Deficiency and of Chronic Dilantin Sodium Toxicity in Rats.
22	M. Rogosa	Immunological, Biochemical and Pathological Studies of Oral Bacteria.
23	H. W. Scherp	Manifest and Inapparent Viral Infections of Oral Tissues.

NATIONAL INSTITUTE OF DENTAL RESEARCH
Annual Narrative and Project Report
Calendar Year 1959

CONTENTS

LABORATORY OF MICROBIOLOGY (Continued)

NIDR No.	Investigator(s)	Project Title
24	H. W. Scherp & S. E. Mergenhagen	Digestion of Native and Altered Collagens by Oral Microorganisms in Relation to Periodontal Disease.
25	T. Shiota	Nutrition and Metabolism of Lactic Acid Bacteria.

LABORATORY OF BIOCHEMISTRY

NIDR No.	Investigator(s)	Project Title
26	F. J. McClure	Nutrition and Dental Caries with Emphasis on Cereal Foods, Lysine, Calcium and Phosphate Supplements.
27	I. Zipkin	Biochemistry and Physiology of the Salivary Glands and their Secretions.
28	I. Zipkin	Non-dental Physiological Effects of Fluoride.
29	H. G. McCann	The Inorganic Chemistry of Dental Tissues, Surface Enamel Chemistry as Affected by Diet and Oral Environment, and their Relation to Dental Caries.
30	J. E. Folk	Protein and Amino Acid Chemistry. I. Proteolytic Enzymes; Chemical and Molecular Studies, Isolation, Purification and Kinetics. II. Blood Clotting; Enzymology, Metabolism and Molecular Mechanism.
31	M. S. Lewis	Physical Chemistry of Proteins.
32	M. S. Lewis	Biochemistry of Salivary Proteins.
33	R. C. Likins	Biochemical and Biophysical Studies of Calcification.
34	K. A. Piez	Analytical and Structural Aspects of Collagen in Relation to Function and Disease.
35	K. A. Piez	Biochemistry of Amino Acids and Proteins in Mammalian Cell Cultures.

[illegible]
[illegible]
[illegible]

LABORATORY OF BIOCHEMISTRY (Continued)

NIDR No.	Investigator(s)	Project Title
36	R. H. Larson	The Role of Tetracycline in Experimental Caries in the White Rat.
37	H. Blumenthal	Metabolism of Oral Flora and Salivary [illegible] (A) Nucleoside Metabolism in Rat Submaxillary Glands; (B) Hexosamine Formation of Microorganisms
38	G. R. Martin	Studies on Collagen. I. Biochemical Studies on Collagenolysis. II. Relation of Amino Acid Composition and Reactivity to Polymer Form.

EPIDEMIOLOGY & BIOMETRY BRANCH

NIDR No.	Investigator(s)	Project Title
39	A. L. Russell	The Epidemiology of Dental Caries - [illegible] the Fluoride-Dental Caries Relationship
40	A. L. Russell & E. C. Leatherwood	Epidemiology of Oral Diseases in Vietnam.
41	A. L. Russell & C. L. White	Relation Between Dental Caries and Periodontal Diseases.
42	A. L. Russell & C. L. White	Epidemiology of Oral Diseases among Alaskan Natives.
43	C. J. Donnelly & N. W. Littleton	Epidemiology of Fractured Incisors.
44	E. C. Leatherwood & J. C. Greene	Epidemiology of Oral Diseases in [illegible]
45	C. J. Donnelly	Study of Periodontal Disease and Caries [illegible] Adventists Families.
46	N. W. Littleton	Epidemiology of Oral Diseases among Ethiopians

NATIONAL INSTITUTE OF DENTAL RESEARCH
Annual Narrative and Project Reports
Calendar Year 1959

CONTENTS

CLINICAL INVESTIGATIONS BRANCH

NIDR No.	Investigator(s)	Project Title
47-c	C.J. Witkop, Jr.	Tri-racial Isolates in Eastern United States.
48-c	C.J. Witkop, Jr.	Hereditary Defects of Enamel and Dentin (Michigan study).
49-c	C.J. Witkop, Jr.	Genetic study in Southern Maryland.
50-c	C.J. Witkop, Jr.	Hereditary eye defects.
51-c	D.L. Rucknagel	Sickle cell anemias and other inherited hematological disorders.
52-c	J.D. Niswander	Oral aspects of the results of consanguineous marriage in Hiroshima and Nagasaki, Japan.
53-c	R.M. Stephan	Studies of the oral microbiota and oral environment in relation to dental and systemic diseases.
54	R.M. Stephan	Interrelationships between local and systemic factors in the etiology of dental diseases in rats.
55-c	A.D. Merritt	Studies on enzymatic reactions of cyclic alcohols and steroids.
56-c	A.D. Merritt	Studies of the urinary excretion products of histidine in patients with liver disease.
57-c	P.J. Coccaro	Serial cephalometric study of the longitudinal growth of the soft palate and nasopharynx in cleft palate children.
58-c	E.J. Driscoll	General anesthesia (dental) baseline data.
59-c	I.I. Ship	Investigations into the etiology of recurrent aphthous ulcerations and other oral nonmalignant soft tissue diseases.
60-c	I.I. Ship	Stress in the dental patient.

[illegible]
Annual Narrative and Project Reports
Calendar Year 1959

CONTENTS

CLINICAL INVESTIGATIONS BRANCH (Continued)

NIDR No.	Investigator(s)	Project Title
61-c	I. I. Ship	Secretory sialography in patients with Sjogren's Syndrome and rheumatoid arthritis.
62-c	I. I. Ship	Effect of dietary phosphate on dental caries in children.
63-c	P. N. Baer	Studies on periodontal dressings. Par[illegible] A hydrogenated fat-zinc bacitracin dressing.
64-c	P. N. Baer	Studies on periodontal disease in the [illegible]
65-c	H. R. Stanley, Jr.	Histopathology of the human pulp.

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

Annual Report on Intramural Research

Calendar Year 1959

I. Introduction

In the simplest terms of definition and viewed in a broad sense, the mission of the intramural program of the National Institute of Dental Research is to contribute with all the means at its command to an ever-expanding knowledge of oral health and disease; this to the end of insuring an ever-improving oral health status of the people. To pursue this goal a variety of resources, including high-caliber scientific manpower and technological equipment, are available; a striking contrast to the early days of dental research when responsibility was primarily in the hands of individual practicing dentists and manufacturers of dental materials.

It has been said that dental research, as well as other areas of biological research, probably begins with such questions as how to treat and how to prevent departures from normal in certain structures and functions. Although this concept encompasses structural components and related physiologic and pathologic processes, it should be emphasized that the broadening scope of responsibility of dental research also counts heavily on the epidemiological, sociological, and physical sciences. Thus, in its total intramural activity, the Dental Institute is concerned not only with the cause and control of dental caries and periodontal disease, but also with a variety of other conditions and malformations of the mouth and adjacent structures which include oral cancer, cleft palate and lip, oral manifestations of systemic disease, and the influences of oral diseases on other organ systems of the body. This program is a far cry from the modest beginning of dental research in the U. S. Public Health Service. Initiated in 1931 as a Dental Hygiene Unit, consisting of a single dental officer, its primary function was to apply principles of epidemiology to a series of community studies on mottled enamel. Very shortly this activity was expanded into a full-scale epidemiological program designed to document the effectiveness of fluoridation as a caries control measure. Beginning in 1936 the dental research program was broadened so as to include animal

studies on the physiology and toxicology of fluoride compounds. Understandably, many of these investigations led away from fluoride and into basic research on the causes and prevention of a variety of diseases involving the teeth and surrounding structures.

Staffed today by 50 principal investigators and approximately 70 supporting personnel (excluding administration and the grants program), the various organizational units of the Dental Institute cover a wide range of research activity, constituting today one of the world's largest centers devoted to the study of oral health and disease. Augmenting this professional staff of biochemists, histologists, anatomists, microbiologists, pathologists, epidemiologists, geneticists and clinical investigators with varied backgrounds of educational and research experience, is a small but effective group of Visiting Scientists from Japan, Denmark, England, and Germany. It is not too optimistic to anticipate that the continued encouragement and support of such collaborative research ventures with outstanding scientists from both the United States and abroad, and the parallel arrangementfor assignment of NIDR scientists to other research centers (currently limited to assignment of one scientist at the National Institute for Research, University of Reading, England, and another at the California Institute of Technology) will bring mutual benefit to all participants, and possibly contribute to earlier major breakthroughs in the oral health field. Exemplifying this international exchange of high-level investigators in key areas of scientific endeavor is the recent temporary appointment of Professor Albert E. W. Miles to our professional staff. Having served many years as Professor of Dental Histology and Pathology at the London Hospital Medical College, Professor Miles brought to our Institute a wide experience on the subject of calcification, and an expert knowledge in the field of forensic medicine which are currently being applied to important research on the identification of age changes in the dentition.

In the Visiting Scientist program, there is demonstrated an additional responsibility to provide preceptorship training of a fellowship type to the fledgling researcher who will be the mature scientist of tomorrow. Several such research associates have received temporary appointments in various sections of the Dental Institute in order to acquire skills in new research techniques and to profit from association with experienced and eminent scientists. Hopefully, the benefits of these experiences will be carried back to the respective home institutions and thereby contribute to a more effective program of inter-institutional and

international cooperation in oral health research. In the fellowship category of the Visiting Scientist program may be listed Dr. Shosaburo Takuma from the Tokyo Dental College who, during his preceptorship tenure with Dr. D. B. Scott, pursued an electron-microscopic study of the early stages of bone development that led to significant observations of the stages of osteogenesis from earliest beginning to the point at which mineralization begins. Another Research Associate, Dr. Ellinor Weiss from the Institute for Physical Chemistry, University of Basel, Switzerland, completed a joint assignment with Dr. F. J. McClure, NIDR, and Dr. H. Eagle, NIAMD, that culminated in the preparation of many new and unusual compounds including the first unsymmetrical peptide of cystine to be synthesized.

In the overall program activity of the Dental Institute, a compromise in research philosophy or methodology is necessarily reached as the respective advantages and disadvantages of so-called directed team research are weighed against the independent worker whose direction and pursuit of leads are limited only by his own imagination and competence. In this atmosphere of scientific freedom, it is essential that periodic review and assessment of progress be made in order to provide a judgment for expansion or curtailment of certain research activities. Because our research to date has demonstrated that the major oral diseases, namely, dental caries and periodontal disorders, are probably related etiologically to a complex multiplicity of factors, a principal effort has been directed toward the isolation of each of the various suspected causal elements. In anticipation of the vigorous and broad frontal attack that would be required to accomplish even a small part of this objective, a number of scientists with varied and specialized skills joined forces. It was in such a manner that, in order to insure the greatest flexibility of research project administration, a new section (Gnotobiotic) was established in the Laboratory of Microbiology. The early demonstration by this study group of the caries initiating role of various strains of streptococci in germfree rats is testimony to the effectiveness with which its members utilized their collective imaginations, perserverence and technical skills.

Somewhat different criteria were considered in establishing another new section on Medical Investigations in the Clinical Investigations Branch. Recognizing the responsibility of the Dental Institute for covering the broad aspects of dental research with emphasis in the area of oral medicine, competent medical personnel were made available to evaluate correlations between

oral and medical illnesses; to collaborate in studies of the general pharmacologic activities of drugs useful in the treatment of dental disease; to make observations of the physiologic actions of preanesthetic and anesthetic agents; and to provide consultation to the Institute's genetics program in correlating pedigree data with physical findings so as to determine modes and linkage of inheritance.

Although for purposes of effective administration, staffing and budgeting, the Dental Institute operates within an organizational framework of laboratories, branches and sections, its principal objective of encouraging and supporting meaningful research can only be met by a day to day conduct of activities governed by program areas rather than departmental lines. An analysis of the overall Institute organization makes it possible to present an outline prospectus of the major program areas constituting this research effort.

1. Genetic Studies

   a) The investigation of hereditary dental and oral defects in humans and their relation to other systemic diseases of hereditary character.

   b) Experimental animal studies to gather additional information on the relation of hereditary factors to caries and periodontal disease.

2. Epidemiological Studies

   a) Field investigations to amplify our knowledge of the relationship between oral diseases and nutrition, aging, sex and other factors.

   b) Continued studies of fluoridation.

3. Studies in the area of Clinical and Experimental Pathology

   a) Histochemical and morphological studies of diseases of the soft and calcified tissues of the mouth.

   b) Microbiological studies of dental caries, periodontal disease and other oral conditions.

   c) Investigation of local-systemic factors in oral diseases (periodontal disorders, aphthous stomatitis, chronic diseases and aging).

4. Germfree Studies

   The utilization of germfree technics for determination of the specific microbial relationships to dental caries and periodontal disease.

5. Nutritional and biochemical studies of dental caries and periodontal diseases.

6. Growth and Development Studies

   a) Clinical observations of the normal pattern of oral and facial development.

   b) Experimental studies on the pathogenesis of cleft palate and other congenital malformations.

7. Non-disease oriented basic research programs

   a) Biophysical, biochemical and physiological studies on calcification, collagen, saliva and blood clotting.

   b) Electron and X-ray microscopic studies on the structure and physical properties of bone and teeth.

   c) Studies on fluoride metabolism.

Anticipating the expanded facilities that will be provided in the new Dental Research Building in FY 1961, considerable attention has necessarily been given during the past year to the formulation of plans for the best utilization of scientific manpower and equipment. Thus, a continuing series of weekly planning sessions has been held with the laboratory, branch and section chiefs of the Institute in order to discuss and define research goals, and outline methods for the fruitful pursuit of these objectives. Obviously, this undertaking has required a careful appraisal of current programs of research so as to establish a baseline upon which to make reasonable assumptions of future trends and to construct and reconstruct along lines of promising productivity.

In parallel fashion, the NIDR Board of Scientific Counselors, charged with responsibility for advising on intramural programs in matters of general policy, particularly from a long range viewpoint,

has given consideration to several important areas. In so doing, the following actions and recommendations have been made.

1. The Board recommended that consideration be given to the NIDR's interest in establishing a low-level radioisotope counting facility at the NIH suitable for strontium-90 assays in foods, excreta, and calcified tissues, in order to make surveys possible on the intake, retention, and elimination of radioisotopes derived from the "fallout". Since, with respect especially to strontium isotopes, the health problem appears to be mainly related to the calcified tissues, the Board recommended further that the low-level counting facility be established as a service function for biological studies.

2. The Board hoped that plans for expansion of the Clinical Investigations Branch will come to fruition in order to provide an increased fund of knowledge on the interrelationships of dental and systemic diseases. This hope was optimistically expressed insofar as the patients in the Clinical Center, and the facilities of the Center, furnish a unique opportunity for human studies in the dental field.

3. The Board observed that the NIDR has an outstanding opportunity to work in the field of growth and development. The facilities and multiple disciplines available at the National Institutes of Health suggest that the Dental Institute might devote a major share of attention to basic approaches to the many problems inherent in studies of growth and development. While also endorsing the pursuit of clinical activities, the Board believed that treatment aspects should continue to be deemphasized.

4. The Board suggested that a fruitful area of research might be initiated through the purchase of cine-fluoroscopic equipment for the conduct of such studies as temporomandibular joint function. The success of this activity would necessarily depend upon the recruitment of a well qualified scientist to supervise the program.

5. With the expansion of facilities that will become available upon the completion of the new building for the NIDR, the Board suggested that consideration be given to a revision of the administrative organization of the Institute in order to allow a better integration of existing programs of investigation with new programs of work soon to be undertaken. Activities in the field of biophysical studies of dental tissues having given important results, there is need to enlarge their scope through the use of newer technical methods, now available, and through the addition to the staff of the Institute of specialists in the examination of calcified tissues with physical methods. On such grounds, the Board advised on the need to establish, as soon as feasible, a Laboratory of Physical Biology.

II. Laboratory Research Activities

During the past year, major attention in the Laboratory of Biochemistry was given to the analytical and structural aspects of collagen, the biochemistry of saliva, mineral metabolism, proteolytic enzymes, and the broad field of experimental caries.

Inasmuch as collagen is the major protein of teeth (dentin), as well as skin, tendon and bone, it may well play a role in the function of these tissues and in the pathological states which involve them. Although the data assembled, to date, are basic and fundamental, there is the ultimate objective of application of such acquired knowledge to the problems of dental caries and periodontal disease. Thus, amino acid analyses by Dr. K. A. Piez on a number of collagens from invertebrate phyla have shown them to resemble vertebrate collagen in that they contain hydroxyproline and hydroxylysine, and one third of the residues are glycine. While in other respects they are quite different (the differences possibly being related to function or structure at the tissue level), similarities are in accord with the requirements of the structure of the molecule. Other significant findings suggest that thermal denaturation is associated with the total content of pyrrolidine rings rather than with the hydrogen bonding by the hydroxy group of hydroxyproline.

In studies on the physiology of the salivary glands and the biochemistry of their secretions, it has been shown by Doctors R F. Seever, I. Zipkin, F. J. McClure and M. S. Lewis that a lysine deficiency in the diet of experimental rats, aside from growth influences, produced no fundamental changes in the glands and no alterations in certain protein constituents of the saliva. Thus, the promotion of experimental dental caries by lysine-deficient diets does not appear to involve either the salivary glands or their secretions. Other biochemical studies of saliva have evolved a reliable method for the analysis of two essential amino acids, i.e., tyrosine and tryptophan, which are currently being evaluated in terms of their possible relationship to disease states within the oral cavity. In a correlative approach designed to study the metabolism of the oral flora as related to the salivary gland, it has been found by Doctors H. Blumenthal and T. Shiota that the hexosamines, which are constituents of salivary polysaccharides, are metabolized by lactobacilli casei with the liberation of ammonia and the formation of lactic acid. Such release of ammonia by the submaxillary gland of the rat is indicative of the presence of adenosine triphosphate, a pyrophosphate, or adenosine deaminase within the metabolic turnover of the gland.

A much favored hypothesis in explanation of the cause of dental caries is an abnormally mineralized dentin and enamel. Repeated studies by H. G. McCann, involving extreme variations in

dietary calcium, phosphorus and magnesium, have shown a significant influence on the proportions of these elements, as well as of carbon dioxide, in the teeth of rats. Of considerable significance is that these variations in the inorganic chemistry of the dentition did not coincide with the incidence or severity of the caries experience. On the other hand, additional data obtained by Dr. F. J. McClure continued to verify the cariostatic effect of certain phosphate compounds, whereas a calcium mineral supplement to the diet has given inconsistent results. The presumed implication of these studies is that some fundamental inhibitory reaction occurs locally, perhaps on the exposed surfaces of the teeth.

Further investigating the role of the oral environment in caries etiology, it has been shown by Dr. R. H. Larson that the disposition of an experimental animal (the white rat) to develop dental caries is dramatically affected by treatment during gestation or during a part of lactation, with a broad spectrum antibiotic, tetracycline. The mechanism of this action appears referable to the oral flora, although its significance extends to the influence of prenatal and developmental environment on oral disease.

Collaborating with the National Bureau of Standards in studies of mineral metabolism, Dr. R. C. Likins showed that the comparative renal clearance of calcium-45 and strontium-89 is presumably affected by the nutritional status of the animal; and that the skeletal metabolism of these tracer elements materially affected their excretion in the feces and urine. While the relative amounts of free and protein-bound Ca^{45} or Sr^{89} appear to be unaffected by inanition, possible differences in the binding of these ions by smaller molecules is being investigated by membrane dialysis techniques.

Demonstrating the essential component of non-disease oriented research in the total institutional activity are the basic studies on protein and enzyme chemistry by Dr. J. E. Folk in collaboration with Doctors K. Laki, J. A. Gladner, Y. Levin and W. R. Carroll of NIAMD. Nearing completion during the past year was the final purification and further elucidation of the chemical structure and essential kinetics of the new pancreatic enzyme carboxypeptidase B. Employing this enzyme as a valuable tool in both natural and chemically-modified protein structure research, there has been gained a better understanding of the biochemistry of blood clotting. This information should open the way to a more systematic pharmacological and clinical approach for the correction of certain abnormal blood clotting reactions.

While this year marked the termination of a fifteen year study of dental caries as influenced by water fluoridation in Grand Rapids, Michigan, investigation continues concerning the non-dental aspects of water fluoridation. Inasmuch as the content

of fluoride in skeletal tissues may be increased with an increased fluoride intake, the stability and variability of this bone fluoride deposition was the subject of study by Dr. I. Zipkin. The several different bones in the body of the experimental white rat varied significantly in their capacity to retain fluoride. Likewise, following withdrawal of fluoride from the drinking water, on the average of 7.5 percent of the bone fluoride deposit in the case of the growing animal, and 11.5 percent in the mature animal, was eliminated in a relatively short period of time.

The research program in oral microbiology continued in 1959 to center on dental caries, periodontal disease, and associated studies in systematic microbiology, microbial physiology, and germfree techniques.

Based on a series of highly significant studies by Dr. P. H. Keyes, Laboratory of Histology and Pathology, in which so-called "resistance" to dental caries was shown to be partly due to the absence of a necessary microflora, a concerted effort was directed toward the further understanding of microbial factors in caries etiology and transmissibility of this disease. In a collaborative study, Dr. R. J. Fitzgerald (Gnotobiotic Section) and Dr. P. H. Keyes demonstrated conclusively for the first time a specific bacterial factor in dental caries. Utilizing various refined techniques, including a mouth prop developed by Dr. A. A. Rizzo, it was found that certain kinds of streptococci isolated from carious lesions in hamsters could be implanted so as to cause caries in previously "caries-resistant" hamsters. Since prior to the experiments, the infecting microorganisms were made streptomycin-resistant, it was possible to isolate and positively identify them during and at the end of the test periods. Thus, the fundamental Koch postulates, which require uncomplicated infection, demonstrable disease, and final recovery of the infectious agent, have been shown unquestionably to apply in the case of caries. It should be noted at this point that the infecting microorganisms are very similar to those previously shown by Dr. Fitzgerald to produce caries in otherwise germfree rats. When "caries-resistant" hamsters were experimentally infected with a variety of other streptococci, lactobacilli, and diphtheroids, no carious lesions developed. The results of these important investigations clearly establish caries in hamsters as a transmissible, infectious disease and encourage renewed search for a specific microbial agent in human caries. In the latter connection, timely new data obtained by Dr. A. Howell, Jr. substantiates the predominance of actinomycetes in certain human carious lesions.

In related investigations on dental caries, it has been found by Dr. H. V. Jordan, Jr. that incorporation of an aldehyde- and ketone-binding compound, sodium metabisulfite, in a cariogenic diet,

reduces the incidence of caries in rats by from 33 to 77 percent, probably by alteration of the metabolism of caries-producing bacteria. Thus, sub-bacteriostatic concentrations of sodium metabisulfite were noted to strongly reduce acid production by growing streptococci in vitro, whereas carbon dioxide and nonacid products such as ethanol increased correspondingly.

Definition of the role of microorganisms in periodontal disease should be facilitated by study of experimental infections with oral microorganisms, as developed recently by Doctors E. G. Hampp, S. E. Mergenhagen, H. W. Scherp and R. R. Omata. Based on these investigations, methods have been evolved to follow the pathogenicity of each of the major kinds of bacteria implicated in periodontal disease, alone and in combination with each other and with ordinarily non-pathogenic oral symbionts. For example, the long suspected but never conclusively demonstrated mutual enhancement (syngergism) of oral fusobacteria and spirochetes was substantiated. Ancillary studies revealed oral microbial products that might mediate periodontitis: (a) classic glucolipid endotoxins were isolated from fusobacteria, spirochetes, veillonellae, and selenomonads but not from anaerobic and viridans streptococci or diphtheroids; (b) spirochetes exhibited protease and peptidase but not mucopolysaccharase activity; and (c) cultures of gingival accumulations were shown for the first time to digest an undenatured collagen.

Since deposition of dental calculus on a microbial matrix is generally regarded as a critical determinant of periodontal disease, it is significant that Dr. P. N. Baer in collaboration with Dr. W. Newton (NIAID) reported the presence of tartar-like deposits on the teeth of germfree mice accompanied by periodontal disease. Subsequently, Dr. R. J. Fitzgerald and E. G. McDaniel (NIAMD) found that calcified deposits on the molar teeth of germfree rats contained the same inorganic component, hydroxyapatite, as human calculus. This experimental situation affords opportunity to evaluate the influence on calculus formation of such factors as diet and host physiology without interference from the as yet uncontrolled oral flora.

Viral studies directed by Dr. H. W. Scherp with material from the Gnotobiotic Section, accumulated evidence of the occurrence in salivary glands but not other tissues of germfree rats of a virus cytopathic for certain kinds of cultured mammalian cells. An apparently similar agent was demonstrated in the salivary glands of conventional rats. Other studies established the basis for a serological grouping of strains of herpes simplex virus.

Studies of the nutritional interactions of the oral flora progressed significantly. Dr. T. Shiota demonstrated the synthesis of a phosphorylated folic acid derivative by oral lactobacilli. Dr. T. A. Nevin showed that the symbiotic stimulation of growth of a spirochete by a diphtheroid depends on synthesis by the latter of the unstable growth factors, thiamine pyrophosphate and acetyl phosphate. Dr. Nevin also determined the pathway of arginine metabolism, which seems to be focal for this spirochete.

During the past year, studies with the electron microscope and related physical tools continued to be directed toward bringing forth further basic information on the formation and calcification of bones and teeth, and on their ultimate structure in the fully mature state. Prominent among the significant accomplishments of Doctors D. B. Scott and M. U. Nylen may be mentioned observations on the manner in which enamel formation takes place, and the collection of increasingly precise data on the exact size, shape and distribution of the crystals in both mature and developing calcified tissues. Based on these studies, it now appears that some of the concepts of amelogenesis, established earlier by optical microscopy, will be subject to revision. For example, there is evidence that (a) the first inorganic crystals appear immediately following matrix deposition, with organic material actually found within the crystals themselves; (b) the enamel matrix may be formed in a complex fashion with interrod matrix appearing between the distal ends of the ameloblasts and with the proximal ends subsequently splitting away as parent cells, leaving the isolated distal portions to change into rod matrix; and (c) the so-called distal terminal bar system is not an artifact, as previously suggested by some investigators, nor is it a true terminal bar system although it may appear as such in the optical microscope. Instead the substance responsible for the optical image is actually identical with the initial interrod matrix mentioned above. A true terminal bar system, on the other hand, is found in the proximal ends of the cells and comprises a morphological structure that makes unlikely any direct contribution of stratum intermedium cells to enamel matrix formation.

Another important facet of the laboratory activity employing biophysical methods has been the wide scope of collaborative studies with investigators from other categorical institutes and from outside laboratories and institutions. Some that might be cited are: (a) studies of calcareous corpuscles in tapeworms (with Dr. T. von Brand, NIAID); (b) development of technics for microradiography of ectopic calcifications and tumor formation (University of Texas); and (c) studies on calculogenesis (Eastman Dental Dispensary, Rochester, New York). These interdisciplinary and interlaboratory activities have been fruitful not only because of the new information yielded but also because of the broadened experience of the workers involved.

As alluded to in a previous section of this report, the opportunity for training of research associates in the fellowship category of the Visiting Scientist Program, as well as the training of guest workers, is a most important segment of the Institute's responsibility. With some trainees terminating their assignments at the close of this year, arrangements have been made for the acceptance of three new young professionals to gain experience in electronmicroscopy.

In other phases of laboratory activity, Dr. M. S. Burstone has made considerable progress in the development of new histochemical techniques which broaden the scope of investigations into the composition and reactivity of various tissues. In collaboration with Doctors G. G. Glenner and D. B. Meyer (NIAMD), a most comprehensive study of neoplastic tissue was conducted which provided significant new information about enzymatic and other changes associated with pathological processes. In addition, Dr. Burstone devised methods which led to (a) the finding of high succinic dehydrogenase activity in osteoclasts associated with active bone resorption; (b) the first demonstration of alkaline phosphatase activity by fluorescence microscopy; and (c) the visualization of mitochondrial activity in tissues.

Other continuing histochemical investigations have focused on the composition and characteristics of normal and diseased connective tissues. Under the direction of Dr. H. M. Fullmer, particular emphasis has been placed on the fibrillar components and other non-cellular constituents of connective tissues which have hitherto not been thoroughly investigated. Thus, the properties of a new type of connective fiber, found last year, have been studied further, and the evidence suggests that the partial resemblance of these fibers to elastic fibers might be explained either on the basis of incomplete maturation or specialized function. Although these activities have been basically planned as an initial step in research on periodontal disease, a most important by-product has come from the application of originally devised methods in a collaborative study (with Doctor L. T. Kurland, H. D. Siedler and R. S. Krooth, NINDB) dealing with amyotrophic lateral sclerosis. In this project it has been found that a high percentage of patients (60 percent) with ALS also demonstrate a connective tissue disorder which has been defined histochemically. This abnormality, found in the skin, is characterized by (a) a distinctive elastosis; (b) an increase of mucopolysaccharide; (c) a degeneration and elastosis of arrector pili muscles; (d) focal areas of apparently regenerated connective tissues; and (e) several characteristic alterations of collagen. Thus, additional information on this little-understood disease has been provided, as well as a possible new diagnostic criterion.

Research during the past year in the field of epidemiology continued its efforts to amplify the descriptive and determinative characteristics of oral disease processes.

Recognizing that the occurrence of periodontal disease is widespread and that it contributes significantly to tooth loss, special emphasis was placed on obtaining information on prevalence

and severity in various population groups. In collaboration with the Interdepartmental Committee on Nutrition for National Defense, the Dental Institute initiated a number of studies to evaluate the nutritional status of selected populations in various participating countries. Working closely with the biochemists, nutritionists, clinical pathologists and physicians comprising the survey teams, Doctors A. L. Russell, N. W. Littleton, E. C. Leatherwood, J. C. Greene and G. E. Sydow completed large numbers of oral examinations in Alaska, Ethiopia, Peru, Ecuador and Viet Nam. Although tabulation and analysis of the considerable data assembled has not progressed sufficiently to allow for general conclusions, a few examples of promising leads and findings may be given. In the Alaska study dental caries was virtually nonexistent in individuals from remote villages, and many of the primitive groups living in these areas were found to be essentially free of periodontal diseases. Although other so-called "primitives" exhibited a uniformly prevalent and severe gingivitis, there was little tendency toward progression into destructive periodontal diseases even in the absence of personal oral hygiene or professional dental care. In contrast to these findings, Eskimos that had lived for some time under relatively "civilized" conditions demonstrated a prevalence and severity of oral diseases quite comparable to those seen in average male population groups within the United States. In the Ethiopian study, a most significant finding was that natives subsisting on a high "natural" carbohydrate diet showed a very low prevalence of dental caries. On the other hand, the prevalence and severity of periodontal diseases were high, with no significant differences as related to sex, ethnic origin or geographic area. Nutritional factors appeared to exert only a minor influence on the periodontal tissues in individuals from this sample, whereas positive associations existed between severity of periodontal disease and oral cleanliness. Presumably, the oral and dental findings in the Alaska and Ethiopia studies, as well as those still to be reported in the other surveys, will be appreciably enhanced in significance when correlations are made with the biochemical, nutritional and medical data.

In another approach to the epidemiologic study of periodontal disease and dental caries, Dr. C. J. Donnelly undertook to evaluate the etiologic significance of familial factors and geographic location. Preliminary findings in this survey,conducted on Adventists families, show a consistently low caries rate among children, regardless of geographic differences. Although a similar absence of association was apparent in the case of periodontal disease, there was a tendency for offspring to reflect the periodontal status of their parents.

III. Clinical Research Activities

As indicated earlier in this report, most oral diseases are controlled by a variety of complex factors which include not only local conditions in the oral cavity, but also the general metabolic pattern for the individual and the environment in which he lives. Thus, the range of clinical studies pursued during the past year has been necessarily broad.

In the general program area of human genetics, a significant accomplishment was the description of a heretofore unrecognized hereditary disease transmitted as an autosomal dominant trait and characterized by a defect of cellular maturation affecting the oral tissues and conjunctiva. The related detailed recording of clinical and laboratory findings by Dr. C. J. Witkop, Jr. and his collaborators illustrates well the fertile areas of research on hereditary diseases which may be opened by broad base field studies. In another study designed to define the oral aspects of the results of consanguineous marriage in Japan, a unique opportunity exists to observe the genetic effects of a specific type of mating pattern which complements the continuous type of inbreeding being studied in the eastern United States. It is reasonable to expect that in the former population, certain recessive characteristics determining normal growth and development, as well as pathological traits, will show up in the homozygous state with increased frequency. Sponsored by the National Academy of Sciences and utilizing facilities of the Atomic Bomb Casualty Commission in Nagasaki and Hiroshima, a team consisting of specialists in such fields as pediatrics, anthropology, hematology and dentistry (Dr. J. D. Niswander, NIDR) has been studying approximately 5,000 offspring of first cousin marriages. Although, to date, no striking difference in the prevalence of known hereditary defects has been found when compared with a control group, the narrative data indicate that between 1951 and 1959 there were significant increases in stature, weight and number of permanent teeth erupted for 6, 8 and 10 year old children. It is apparent that this type of information on growth and development is vital to a realistic quantitative appraisal of such problems as are posed by the increasing exposure of the human species to ionizing radiation.

Another promising area of clinical investigation has been concerned with the identification of various external and environmental etiologic factors involved in the pathogenesis of recurrent oral mucous membrane ulcerations. Although a considerable body of evidence is available to support a viral etiology of recurrent aphthous-like lesions of the lips, no such evidence applies in the case of recurrent intraoral canker sores. In collaboration with

the University of Pennsylvania, a student population of 1,039 males and 749 females has been under study by Dr. I. I. Ship to determine the pattern of patient experiences with the two types of recurrent ulcerations. At the end of the first year of study several interesting observations may be noted: (1) recurrent aphthous lesions occurred in 52-57 percent of the study group with a statistical difference between the sexes; (2) recurrent herpes labialis occurred in 38 percent of the group with no notable sex difference; and (3) based on expected and observed proportions of students having both diseases, there appears to be a common etiology in the case of males. This correlation has not yet been established in the female group.

With the increasing activity in clinical investigations during 1959, related principally to expansion in the genetics program and greater attention to the general field of stomatitis, justification was seen for the establishment of a Medical Investigations Section. The functions of this professional group of two physicians and supporting personnel, under the direction of Dr. A. D. Merritt, were described in a previous section of this report. As indicated, a major responsibility has been to give consultation in studies of the general pharmacologic action of drugs useful in the treatment of oral disease, particularly related to physiologic response to preanesthetic and anesthetic agents. In this regard, Dr. E. J. Driscoll, in collaboration with Dr. G. R. Christenson, Anesthesiology Department, Clinical Center, has been engaged in a study of alterations in physiological mechanisms following use of various barbiturates including sodium methahexital, neraval, and pentothal. Sufficient data is now available to determine objectively from brain wave activity the precise anesthetic levels at which various operative procedures are carried out. In general, the anesthetic plane is extremely light for all three drugs. With baseline data such as this, the profession is in a better position to improve and refine present methods of anesthesia and lay a sound foundation for further anesthesiology research.

In another activity having practical application for the dental profession, Dr. H. R. Stanley, Jr. has demonstrated a favorable pulp tissue reaction to the newly introduced techniques of high rotary speed instrumentation for surgical removal of normal and diseased enamel and dentin. Emphasizing the important interrelationships of rotary speed, adequate cooling at the site of cutting, and light load of operating pressure not to exceed eight ounces, this clinical study has furnished a basis for the practitioner to better understand the uses and abuses of a new tool in operative dentistry.

With the rather diverse approach to clinical research exemplified by the foregoing activities, and the demonstration by Dr. R. M. Stephan and his collaborators that the development of caries and periodontal disease results from the interaction of many different factors including hereditary predisposition, physical properties and nutritional value of the diet, and retention of food and growth of microorganisms on the teeth, it is apparent that a broad base of knowledge is being assembled upon which to build an increasingly productive program.

- - - - - - -

While it has often been said that dentistry and dental research are part and parcel of the total broad field of biology and medicine, relatively few examples may be cited where this unity of purpose exists to the virtual exclusion of all barriers of traditionally vested interests and conflicting philosophies of purpose. In a research institution devoted to a common objective of learning, there prevails a sense of skepticism, spirit of inquiry and dedicated responsibility to seek for new knowledge that makes for a most unique environment. With the further asset of a multiplicity of disciplines and scientific talents which encourage intellectual cross-fertilization, the National Institute of Dental Research faces the challenge of the future with optimism.

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

Annual Report on Extramural Research

Calendar Year 1959

Research Grants Program

While basic sciences such as biochemistry, morphology, pathology, and physiology, because of their fundamental importance, continued to remain prominent in terms of the number of projects supported, programs of special interest were given considerable emphasis and included periodontal disease (clinical and non-clinical), cleft lip and palate (congenital anomalies), dental caries, conferences and symposia, epidemiology, radiation, aging, and electromyography.

As one of the more serious problems of national oral health, cleft lip and palate and associated anomalies were discussed throughout the year in relation to roentgenographic analyses, diagnosis, operative and post-operative and rehabilitative procedures. Evaluation of the cleft palate programs supported by NIDR has led to recognition of the need for more emphasis on basic research and training in these areas. Efforts to stimulate basic research in the cleft palate field have resulted in the approval by the Dental Council at its June 1959 meeting of the following resolution:

> "The Council has previously expressed an interest in supporting research in the field of cleft palate research and investigations in other areas of speech defects. Because of increasing interest and the development of greater activity in cleft palate and speech rehabilitation centers, the opportunity for investigation of problems related to these defects is broadening."
>
> "The Program Planning Committee recommends that the Council give consideration to setting aside $200,000, identified for support of research in problems related to these fields."

Additionally, NIDR supported the International Symposium on Congenital Anomalies of the Face and Associated Structures. This symposium which was held December 6-9, 1959, at Gatlinburg, Tennessee, considered prenatal development, genetic and metabolic factors, experimental teratology, postnatal growth and development of the head, anatomy and physiology, and clinical investigations. The objectives of the symposium were the promotion of knowledge and the stimulation of broad research programs.

In the oral health field periodontal disease is of major importance since it is primarily responsible for the loss of teeth in persons over the age of 35. The etiology of periodontal disease is virtually unknown, damage to the periodontal tissues is irreparable, and treatment of the disease is empirical. In an effort to gain a better understanding of this disease, basic studies have been expanded, for example, an award was made for a five year grant ($39,772 per year) to investigate the biosynthesis of collagen in periodontal tissues. Since periodontal disease is primarily a degenerative disease involving collagen, it is believed that basic knowledge of the disease etiology will result from investigations of those factors contributing to the destruction of the periodontium.

Another equally significant development in the periodontal disease area was the approval of a five-year grant totalling $175,000 for a comprehensive study of dental calculus. This program grant supports an integrated, multidisciplinary approach to the factors responsible for the formation of calculus and the development of safe methods for its prevention or removal.

Concern over the need for more studies on the epidemiology of periodontal disease motivated the Council to seek the assistance of the Public Health Research Study Section in developing methods for better investigational design of such studies. The lack of dentally trained personnel available for epidemiological work was emphasized.

The development of most oral diseases is controlled by a variety of intricate factors which involve the entire organism. In an effort to promote more research in the field of genetics, plans were completed in 1959 and funds were awarded to support a symposium on genetics related to oral health. It is the purpose of the symposium to define three fields of genetics; 1) recent developments in medical genetics, 2) heritable diseases of dental interest, and 3) genetic methods applicable to the investigation of intrinsic dental disorders. Publication of the proceedings of the conference will provide dental researchers with comprehensive source material in the field of genetics as related to dental interest and establish a basis for further research.

NIDR is interested in the phenomenon of aging as related to oral health, since all living cells, tissues, organs, and the total organism change progressively with the passage of time. One example of a basic research study with an aging component is an approved three year grant amounting to approximately $119,000. This phase of the study is directed to a better understanding of the organization structure and function of mineralized tissue, as well as the intimate details of the mineralization process itself. There is little doubt that disturbances in the process of calcification of the organic matrix are important in almost every field of medicine and surgery. In order to understand the pathogenesis of these varied pathological conditions which are characterized by abnormal calcifications, basic information on the factors involved in normal calcification is desired.

Another study directed to an understanding of the calcification process as related to oral health has continued this year. This investigation involves research on the effect of long chain polymers on nucleation of hard tissue apatite; the structure of hard tissue and allied chemical compounds by x-ray diffraction; and the linkage between apatite crystals and collagen in calcified tissue. Specifically, the study is directed not only to a better understanding of the calcification process, but additionally, the role of collagen in nucleating the apatite in bone and teeth, which is yet unknown, will be investigated. Further, basic knowledge of the long range periodicity characteristic of collagen which is essential to the mechanism of mineralization will be sought.

The grant also provides for the training of dental researchers in crystallographic techniques, thus providing a trained nucleous of workers competent to conduct independent research in the field when they return to their parent organizations. Total support of the grant which is now in its third year amounts to approximately $18,000 per year.

The ten state study of the status of radiation hygiene in dental offices was initiated last year at the request of the Council for an integrated and concentrated research effort to determine the extent of possible hazards associated with present programs involving clinical x-ray procedures. The study is unique in that in addition to the ultimate value to the dental profession, it involves ten identical research procedures conducted under rigorous standardization both as to methodology and equipment.

It is anticipated that a critical analysis of the data resulting from the study will provide information which will be useful in reducing genetic and somatic effects of radiation to patient and operator. Current NIDR support of this program amounts to approximately $17,000. The study is scheduled for completion in 1960.

During 1959 the number of active research grants totalled 369 in 35 states and 6 foreign countries as compared to 350 research grants in 32 states and 4 foreign countries last year. The appropriation in 1960 included an increase of $1,032,000 for a total of $4,476,000. The approval rate for all new applications reviewed in 1959 continued at a level of approximately 50%.

Future planning includes active programming in three broad areas: 1) periodontal disease, 2) oral congenital anomalies (cleft lip/palate), and 3) dental caries. Additional emphasis will be concentrated on other important facets of dental research such as: chronic disease oral-systemic interrelationships, electromyographic studies of the jaws, dental radiation investigations, aging studies, and dental public health research.

Research Fellowship Program

The National Institute of Dental Research fellowship program offers four types of awards; 1) dental student part-time fellowships; 2) predoctoral fellowships; 3) postdoctoral research fellowships, and 4) special research fellowships.

The primary purpose of the research fellowship program is directed toward graduate training in the basic sciences. Special emphasis has been placed on increasing the number of trained individuals in the basic sciences related to the study of periodontal disease, cleft palate, and dental caries. In dental schools today there is a shortage of teachers trained in research. As the schools broaden their curricula and increase or better their research facilities, this shortage becomes more acute. The Institute has recognized the need of competently trained personnel and has sought to support sound research training, to encourage training in critical disciplines, and to anticipate the demand for support from the growing number of graduate students.

Dental Student Part-Time Fellowship Program

The Division of General Medical Sciences is the administrative agency responsible for the Dental Student Part-Time program while the NIDR Extramural Programs Branch retains responsibility for programming and budgetary needs. Increased appropriation for fellowships in fiscal year 1960 has made possible an increase from six to eight training units per dental school. All 47 dental schools are now participating in the program. The total budget expended for this program in 1959 was $244,000 for 376 training units.

A uniform beginning date for all Dental Student Part-Time awards has been established with a December 1 deadline for applications.

A preliminary study of the student part-time program made this year shows that a high percentage of the participating students have continued their research training by receiving support from the Post-Sophomore or Postdoctoral Research Fellowships or as trainees under the Training Program. This worthwhile program is being expanded further by offering two units to each of the ten Schools of Public Health. By this means students drawn from dental schools may receive vital research experience in Public Health during the summer months.

Regular Fellowship Program

During the year the Extramural Programs Branch assumed responsibility for all aspects of the regular fellowship program except receipt and preliminary review of applications. A total of 48 awards were made in the amount of $319,354. In comparison, last year a total of 46 awards of regular fellowships were made at the level of $225,000. The increased cost in 1959 was due to the increase of stipend and dependency allowances

effective January 1, 1959. Graduate students under this program are training in such basic sciences as anatomy, biochemistry, pathology, physiology, histology, enzymology, etc., and in the clinical sciences of periodontia, pedodontia, and orthodontia.

Training Grant Program

Efforts given the graduate training program through the transfer of $200,000 (FY-1959) from the research grant program have resulted in the activation of six new training centers bringing the total number of active training centers in 17 states to 22, as compared with 16 centers in 12 states in calendar year 1958. The increase in the training grants budget from $650,000 in FY-1959 to $1,100,000 in FY-1960, permitted the payment of all but three of the previously approved requests for training.

There are three general types of training grants designed to establish 1) relatively broad research training centers where multidisciplinary programs could be set up, 2) programs in only a single area of training because of the availability of important facilities in terms of research and personnel, 3) smaller grants to places with less research potential to enable younger faculty members to go elsewhere for special training.

Currently there are 34 active training grants supporting 110 trainees at 26 training centers. Of the total number of training grants, 26 were awarded to training centers, and eight were given to those schools not designated as training centers, so they could send students to training centers of their choice for special training.

One example of specialized research training involving both a university training center and an affiliated but separate facility where NIDR supported research is underway is the Lancaster Cleft Palate Clinic. One trainee is spending a year at the Clinic devoting his efforts to research training in the comprehensive diagnosis, treatment, and rehabilitation of the cleft palate patient. Although the Clinic has received widespread recognition and approval of its integrated program for the treatment of the cleft palate patient, this year marks the initiation of its formal research training program. The program is of special interest to the dental profession and NIDR since student selection is based in part on demonstrated interest in teaching and research in dentistry.

The primary objective of the NIDR graduate training program is to increase the number of competent researchers and/or teachers in dental schools and other dental research institutions throughout the United States. The program is designed to meet the critical shortage of clinical and basic science personnel presently available to these institutions. Increased physical facilities are being built by many dental research institutions. At least ten new dental schools are projected during the next ten year period. These will require substantial numbers of additional personnel for research and teaching.

Notwithstanding the current increase in training budget, it is estimated that our training program is falling short of meeting any reasonable demand. At the current rate of training it would require 12 years to double the full-time equivalent manpower in dental schools, yet during the next ten years the expected needs for new dental teachers with research training has been estimated at 650 per year, or approximately two times the current full-time equivalent figure.

Recognizing the dental manpower problem and the potential of increasing the quality of dental services by the effective use of dental ancillary personnel and facilities, the Council accepted a recommendation from the Training Grant Committee that consideration be given to awarding grants which would provide funds directly to dental schools for the training of dental students in the use of dental assistants.

It is proposed that such grants be based upon the experience of the pilot studies conducted at selected dental schools over the past three years by the Division of Dental Resources, together with information developed through the cooperation of the ADA Council on Dental Education, the American Association of Dental Schools, and the Public Health Service. To provide means to meet the expected target dates of such grants (1960-1962 academic year) conferences have been organized to outline the plan for implementation of the program, and to establish criteria for evaluation of the program periodically on a nation-wide basis.

The success of the Program Directors' meeting held in 1958 initiated requests from training officials for a similar meeting in 1960 to again consider : 1) the exchange of ideas relevant to future dental training; 2) determination of manpower needs in various areas; and 3) coordination of dental training activities on a national scale.

THE NATIONAL INSTITUTE OF DENTAL RESEARCH

Annual Report of Administration

Calendar Year 1959

During 1959 two new sections were added to the NIDR organizational structure. They were the Gnotobiotics Section under the Laboratory of Microbiology, and the Medical Investigations Section of the Clinical Investigations Branch. A description of these activities is included in the research project report.

The NIDR Building Planning Committee, working in conjunction with members of the Research Facilities Branch, completed final layouts for the new dental building, scheduled for completion in the spring of 1961. Additional time was devoted to cost analysis of the glass and cage washing operation planned for the new facilities. Other studies of requirements for movable equipment and a forecast of increased staff needs also were completed during the year.

Beginning July 6, NIDR provided a three-month training assignment for one NIH administrative trainee, marking the fourth such assignment since the inception of the NIH Training Program. In addition, the Institute recommended a sub-professional employee for training under PL-85-507, thereby extending its interest in training of staff members.

Several portable items of dictating equipment were purchased for use in field studies being conducted on a continuing basis in various parts of the United States. Such equipment enables the physician or dentist to record his comments during the examination, thus effecting a savings in manpower and time since such equipment eliminates a need for a human recorder to take notes. Equipment was selected to match that used in the Clinical Center Medical Records Department in order that the investigator can transmit this medical history data on disks and submit them directly to that organization, thereby eliminating the necessity of redictating the material.

Administration of the Extramural Programs Branch was strengthened during the year by the addition of two professional people to the staff. 1) Dr. Kenneth L. Potter, D.D.S., M.P.H., joined the staff of the Extramural Programs Branch as Assistant Chief. Dr. Potter is responsible for administering the Training Grant and Fellowship programs. 2) Mr. Raymond A. Morrison, staff assistant, reported for duty in December. He will undertake categorical and statistical program analyses.

An important administrative development during 1959 was the adoption of a card system for the categorical analysis of the research grants program by areas and disciplines. This system classifies all

dental research under eight broad areas: 1) Basic, 2) Applied, 3) Clinical, 4) Non-Clinical, 5) Disease-oriented, 6) Non-disease oriented, 7) Epidemiological, 8) Educational. These several areas are subcategorized into the clinical sciences, basic sciences, and diseases/conditions.

NIH's initiation of the teleticketing system for obtaining airline tickets has imposed some additional work for the Institute's secretaries, and the Administrative Office has recommended to NIH Management that these activities be centralized within the Office of Administrative Management in a manner similar to that formerly offered by PHS.

A field station in rented quarters at Brandywine, Maryland, used by the Human Genetics Section, was closed during the year. All personnel and equipment was assigned to the Clinton, Maryland field office, thereby effecting a saving in manpower, property, and funds.

Serial No. NIDR-1
1. Office of Associate
Director
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Prenatal Influences on Fetal Development.

Principal Investigator: Dr. S. J. Kreshover

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	2 1/5
Professional:	1/5
Other:	2

Project Description:

Subproject A:
Objectives:

To study the effect of uterine circulatory arrest on the development of the rat fetus.

Methods Employed:

Anoxia was induced by a temporary arrest of the maternal blood supply to one uterine horn in each of 249 gravid Sprague-Dawley rats for periods of 10 to 60 minutes during stages of pregnancy from 7 to 17 days.

Major Findings:

1. Palatal development was affected by interference of circulation to the placenta, particularly when occlusion occurred on the fourteenth or fifteenth day of gestation. Of the 871 viable experimental young that were examined just prior to expected day of delivery, 26 were grossly abnormal (13 with palatal clefts; 16 having limb deformities; one showing an exencephaly; and one being grossly misshapen).

2. The incidence of fetal death was significantly related to the duration of circulatory interference; the greatest incidence (83 percent) being after one hour of occlusion on the thirteenth gestational day.

Subproject B:

Objectives:

To study the influence of external pressure on fetal development.

Methods Employed:

1. Gravid rats, ranging in gestational development from 13 to 16 days, had their uteri exposed through a midline abdominal incision and a fine needle puncture made through the amniotic sac of each embryo in the right or left uterine horn so as to cause loss of amniotic fluid. The opposite, non-punctured horn provided the source of control fetuses.

2. Uterus implantation sites at varying stages of embryonal development were enveloped in closely adapted rigid molds for varying durations of time. In order to assure close adaptation, the ticonium molds were previously fabricated from impressions taken of uteri in all stages of gestational development.

Major Findings:

1. Cleft palates and limb deformities were found in 0, 5, 33 and 6 percent respectively of the offspring of rats subjected to amniotic puncture at 13, 14, 15 and 16 days of gestation.

2. Fetal resorptions were highest (60 percent) following amniotic sac puncture on the fourteenth gestational day; and considerably lower at 16 days (7 percent), 15 days (28 percent) and 13 days (38 percent).

3. Studies on the effect of confinement of the developing embryo and amnion in a rigid mold are still in an exploratory phase.

Significance to Dental Research:

Although congenital abnormalities of the oral structures, including the palate, teeth and supporting tissues, have been the subject of considerable attention, much remains to be learned of the various etiologic and contributing factors involved.

Proposed Course of Project:

Further exploration of maternal factors influencing fetal development.

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1959

HISTOLOGY & PATHOLOGY:

2. D. B. Scott and M. U. Nylen: Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.

3. M. U. Nylen and D. B. Scott: Studies of the Structure of Developing Calcified Tissues by Electron Microscopy and X-ray Microscopy.

4. D. B. Scott and M. U. Nylen: Secondary Collaborative Projects and Training Activities.

5. A. E. W. Miles and D. B. Scott: Age Estimation on the Basis of Histologic Examination of Teeth.

6. S. Takuma: Electron Microscopic Studies of the Ultrastructure of Developing and Mature Bone and Dentin.

7. P. H. Keyes: Experimental Animal Caries.

8. M. S. Burstone: Diagnostic Oral and Surgical Pathology Utilizing Histochemical Techniques with Emphasis Upon Periodontal Disease and Neoplasia.

9. M. S. Burstone: Development and Application of Histochemical Methods in the Study of Hard and Soft Tissues.

10. H. M. Fullmer: Histochemical Studies of Connective Tissues.

Serial No. NIDR-2
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the Structural, Physical, and Chemical Properties of Calcified Tissues by Electron Microscopy and Electron Diffraction.

Principal Investigator: Dr. D. B. Scott

Other Investigators: Dr. M. U. Nylen

Cooperating Units: None

Man Years (calendar year 1959):

Total:	1 3/4
Professional:	1/2
Other:	1 1/4

Project Description:

Objectives:

1. Determination of the crystal structure of enamel by electron microscopy and electron diffraction.

2. Investigation of the reaction of various chemical agents, such as fluoride compounds, on tooth surfaces, with emphasis on the mechanisms by which acid-solubility of enamel can be altered.

Methods Employed:

Positive collodion-carbon replicas and pseudoreplicas are made from the outer surfaces of teeth and from the surfaces of ground sections. The specimens are pretreated in various ways, including simple polishing, etching with acid, and removal of organic constituents by treatment with suitable solvents. Replicas are examined under the electron microscope and the crystalline material retained on pseudoreplicas is in addition subjected to electron diffraction.

Major Findings:

1. The principal effort this year has been directed toward the study of crystal structure of enamel as revealed by removal of the organic matrix. This has been carried on through treatments of ground sections with organic solvents such as ethylene diamine and sodium thioglycollate, and study of the consequently liberated crystalline material by means of replication techniques. Suggestions continue to be found that the basic particle size in mature enamel falls in the range of 300A, and that these particles are aligned in a linear fashion established by the configurations of the organic matrix. Additional evidence indicates that organic material may actually be found within the crystals themselves.

2. Preceding work in this Laboratory indicated that the action of stannous fluoride on enamel differed from that of sodium fluoride in (1) that a much more pronounced protection against acid etching was afforded and (2) that the mechanism of protection was not through calcium fluoride formation (which results from treatment with NaF) but through production of a surface layer of hydrous hydrated stannous oxide. The properties of this protective layer have now been studied in greater detail, and data have been accumulated on its resistance to water and acid solution, as well as rate of disappearance on application of abrasives.

An additional facet of the studies on fluoride effects has been augmentation of an optical microscopic investigation of the surfaces of teeth showing chronic endemic fluorosis with electron microscopic examinations. The optical work was completed several years ago, but the electron microscopy has had to await the development of suitable techniques. The conclusions from this study indicate that, except in the case of pits, the structural differences between fluorosed and normal enamel are extremely subtle. This is in marked contrast to the obvious grossly visible differences, which may thus be due to chemical or physical properties not strongly reflected morphologically.

Significance to Dental Research:

The present work should help fill in some of the facts needed for basic understanding of the normal composition of enamel and its reaction on exposure to fluoride compounds.

Proposed Course of Project:

Continuation along same lines, with added emphasis on correlation with other parallel studies on growth and development.

Part B. included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publication other than abstracts from this project.

Scott, D. B. and Nylen, M. U. Changing Concepts in Dental Histology (accepted for publication, Annals N.Y. Acad. Sci.)

Pedersen, P.O. and Scott, D. B. Replica Studies of the Surfaces of Teeth Showing Evidence of Chronic Endemic Fluorosis. Deut. Zahn-, Mund-, und Kieferheilk. 31:12(1959)

Honors and Awards relating to this project:

None

Serial No. NIDR-3
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the Structure of Developing Calcified Tissues by Electron Microscopy and X-ray Microscopy.

Principal Investigator: Dr. M. U. Nylen

Other Investigators: Dr. D. B. Scott

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2
Professional: 3/4
Other: 1 1/4

Project Description:

Objectives:

1. Study of enamel formation including the submicroscopic morphology of the enamel-forming cells and the organic and inorganic components of enamel.

2. Investigation into the mineral-collagen relationship in calcifying turkey tendons.

Methods Employed:

Specimens are fixed, dehydrated and embedded by special methods. Thin sections are cut on a Porter microtome or a modified Spencer microtome and studied in the electron microscope. A number of sections are shadowed with palladium following removal of the embedding medium. Thicker sections for X-ray microscopy are cut from identical blocks and are mounted directly on the photographic emulsion.

Major Findings:

1. Amelogenesis: Further studies have indicated that the enamel matrix may be formed in a somewhat more complex fashion than hitherto suspected. First, interrod matrix substance is formed between the distal ends of the ameloblasts. Second, the part of the ameloblast surrounded by the interrod matrix appears to be cut off from the parent cell by the formation of a double membrane structure at the most basal point of the interrod matrix, and third, the now isolated portion of the cell is transformed into enamel matrix. The process is continuous, with one segment forming on top of the other until the enamel has reached its predestined thickness. A concept like the one outlined above would explain the herringbone pattern in rodent enamel and also the cross striations observed in mature human enamel.

   As a consequence of this study of enamel matrix elaboration, information has been obtained regarding various points which have been in dispute in previous work with both optical and electron microscopes. Of primary concern has been the question of terminal bars. Over the years the presence of two terminal bar systems, one at either end of the ameloblasts, has been alternatingly confirmed and denied. With the advent of electron microscopy the actual structure of the terminal bar has been shown in studies of other epithelial cells. Based on these conclusions the presence of a terminal bar system in the basal end of the ameloblast has now been confirmed. The structure observed optically at the distal end of the cell has proved, however, to be the interrod matrix mentioned above with no similarity structurally to a terminal bar system. The basal terminal bars represent points of intimate cellular contact and their presence in this part of the cell makes it somewhat unlikely that the stratum intermedium cells should contribute directly to the enamel matrix formation as has been claimed by some investigators.

   In addition to the basal terminal bars intercellular bridges, as defined by electron microscopic observations in other tissues, were found to exist between the basal ends of the ameloblasts, between the ameloblasts and the stratum intermedium cells, and in greatest number between these latter cells.

2. The study on the calcifying turkey leg tendon has been continued with special emphasis placed on the morphological collagen-mineral relationship. Crystallization appears to begin on the surface of the collagen fibrils with the crystals arranged without special reference to the collagen cross banding. Consequently the crystals are found inside the actual fibrils in a very orderly fashion. Close scrutiny of both shadowed and unshadowed sections seems to indicate that the first crystals are placed on the collagen interbands. The cross banding which becomes enhanced during these initial stages of mineralization due to this orderly arrangement of the crystals along the interbands represents a reversal of the original collagen cross banding, the interband regions now appearing denser than the bands. Two types of crystals, platelets and so-called needles are observed. The platelets can only be differentiated at the earliest stages of mineralization while the bulk of crystals at later stages appears to be of the needle type. Using selected area electron diffraction it is possible to show that both types of crystals are oriented with their c-axes parallel to the fibril direction.

Significance to Dental Research:

A knowledge of the cellular processes involved in the formation of the enamel is necessary to understand completely the changes which may result from systemic disorders during development. A study of the ultrastructure of the organic and inorganic portion of the mineralized tissues themselves is important for the same reason and also for the understanding of pathologic involvement after maturation.

Proposed Course of Project:

Continuation of the study of developing enamel. Also an investigation by replicas of ground sections of mature human enamel treated with various organic and inorganic solvents in order to bring out the cross striations of the fully developed prisms. In addition studies of cellular changes in odontogenic cells from animals subjected to a selected systemic disorder of either nutritional, toxic, or infectious origin will be initiated.

Part B. included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Nylen, M.U. and Scott, D.B. and Mosley, V.M. Mineralization of Turkey Leg Tendon. II. Collagen-Mineral Relations Revealed by Electron and X-ray Microscopy. (accepted for publication, Monograph by A.A.A.S.)

Nylen, M.U. and Scott, D.B. Electron Microscopic Studies of Odontogenesis. (accepted for publication, Oral Surg., Oral Med. & Oral Path.)

Nylen, M.U. and Scott, D.B. Basic Studies in Calcification. (accepted for publication, Oral Surg., Oral Med. and Oral Path.)

Scott, D.B. and Nylen, M.U. Improved Preservation of Tendon for Electron Microscopy Through Wet Freeze-drying. Nature 183:1205 (1959)

Scott, D.B. and Nylen, M.U. Changing Concepts in Dental Histology. (accepted for publication, Annals N.Y. Acad. Sci.)

Honors and Awards relating to this project:

None

Serial No. [illegible]
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Secondary Collaborative Projects and Training Activities.

Principal Investigator: Dr. D. B. Scott

Other Investigators: Dr. M. U. Nylen

Cooperating Units: see below

Man Years (calendar year 1959):
Total: 2 1/4
Professional: 1/2 (Scott 1/4; Nylen 1/4)
Other: 1 3/4

Project Description:

Objectives, Methods Employed and Major Findings:

During the present year a relatively large number of requests has been received for applications of biophysical methods to problems under investigation in other laboratories. It has long been accepted that such operations as electron microscopy cannot be adapted to service function, primarily because each type of specimen to be studied presents new and usually difficult problems in handling, and because the microscopic observations must be made by an experienced professional investigator. Inasmuch as many of the requests represent worthwhile problems, and since most relate in some fashion to the general program of this laboratory, the staff has undertaken to offer as much secondary collaboration as seems feasible. The magnitude and success of these projects, listed below, have reached a level at which it is now necessary to consider this activity as a regular part of the Laboratory program.

1. Studies of calculogenesis; primarily electron microscopy and diffraction of intraoral deposits formed in vivo on polyester strips attached to teeth. With Dr. H. A. Zander, Eastman Dental Dispensary, Rochester, New York. To be continued; first manuscript submitted for publication.

2. Study of dental calculus isolated from antibiotic-treated and germ-free rats; electron microscopy, and electron and X-ray diffraction of scrapings, suspensions and thin sections. With Dr. R. J. Fitzgerald, Laboratory of Microbiology, NIDR. To be continued; first manuscript submitted for publication.

3. Investigation of the effects of microbial and chemical treatment of reconstituted collagen, related to experiments on connective tissue inflammation; electron microscopy of micro-drop suspensions and sectioned material. With Dr. S. E. Mergenhagen, Laboratory of Microbiology, NIDR. To be continued; first manuscript submitted for publication.

4. Study of the calcareous corpuscles in tapeworms; electron microscopy, X-ray diffraction, electron diffraction and microradiography of isolated corpuscles and sectioned specimens, for purposes of determining composition and morphological characteristics. With Dr. T. vonBrand, Laboratory of Tropical Diseases, NIAID. To be continued for a short period; first manuscript in preparation.

5. Development of technics for microradiography of salamander embryos, to be applied in studies of calcification and tumor formation; considerable was learned along general lines of embedding and sectioning for radiography. With Dr. B. M. Levy, Dental School, University of Texas. Essentially completed; manuscript on technique submitted for publication.

6. Measurement of dimensions and demonstration of morphological outlines of apatite crystals which show chemical reactivity that varies with size; electron microscopy. With Dr. R. C. Likins, Laboratory of Biochemistry, NIDR. Completed; data and micrographs provided for publication.

7. Examination of ultrastructure of epithelial cells in oral mucosa affected by a newly found genetically determined condition; electron microscopy. With Dr. C. J. Witkop, Clinical Investigations Branch, NIDR. To be continued.

8. Preparation of ground sections of jaws and teeth from tetracycline-treated animals for fluorescence microscopy; involved adaptation of methacrylate embedding method and slicing and grinding techniques. With Dr. I. Zipkin, Laboratory of Biochemistry, NIDR. Completed.

9. Quantitative estimation of poliomyelitis particles by spray droplet counting; preparations were submitted for electron microscopy, and it was found that the proposed methods or modifications thereof did not give accurate results, although some indications of concentration and purity were obtained. With Dr. J. E. Darnell, Laboratory of Infectious Diseases, NIAID. Completed.

In addition to this collaborative work, personnel of this Laboratory were called upon to provide training of various types for a number of individuals (all Guest Workers):

1. Dr. E. P. Cohen, Laboratory of Infectious Diseases, NIAID. Training in all aspects of electron microscopy, including tissue preparation, sectioning, and care and use of the electron microscope. Also, general supervision of research project on effects of amino acid nutrient depletion on the ultrastructure of tissue culture cells. By agreement with Dr. Eagle, the above training is provided by this Laboratory and an electron microscope is made available by NIAMD. Period of training; one year, to end 7/1/1960.

2. Miss M. Pavlovich, Instructor, Department of Bacteriology, University of Arizona. Two months training in embedding and sectioning of tissues for electron microscopy.

3. Dr. W. E. Dossel, Asst. Prof., Department of Anatomy, University of North Carolina. Two week period of training in sectioning for electron microscopy.

4. Dr. H. Benkow, Eastman Dental Dispensary, Rochester, New York. One week period of training in manipulation of microradiographic apparatus.

Significance to Dental Research:

The importance of making collaborative effort available is self-evident, as is the benefit derived by those receiving training. In addition, it is felt that opportunities are provided to the staff of our Laboratory, professional and technical, both for broadening their experience in the general field of biophysical instrumentation and for contributing useful information related in most part to our interests in the oral tissues.

Proposed Course of Project:

As indicated above collaborative work will continue on several problems, and, if feasible, others will be added. In the matter of training, commitments have already been made to one individual from Rochester, N.Y. (Eastman Dental Dispensary) for a three week period, to a worker from Australia (Univ. of Adelaide) for a three month period, and to one from the University of Iowa, for a three month period. All of these personnel are to be supported by their home institutions, and will be classified as Guest Workers at NIDR. It has become evident that if this program is to function effectively, it is a matter of some urgency that one more staff member be added. The type of individual required is most likely an intermediate level professional, with a working knowledge of histology, and, if possible, electron microscopy. We feel that real opportunities for development exist for a young worker, not yet established to the point of specialized interest, who would profit by the varied associations offered.

Part B. included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Four manuscripts recently submitted from this laboratory, one to be submitted shortly, with personnel from this laboratory as co-authors.

Serial No. NIDR-5
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Age Estimation on the Basis of Histologic Examination of Teeth.

Principal Investigator: Dr. A.E.W. Miles

Other Investigators: Dr. D. B. Scott

Cooperating Units: None

Man Years (calendar year 1959)
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives:

For the past fifteen years this Laboratory has been interested in the changes in surface and internal structure of teeth with advancing age. These alterations are of basic biological interest because they relate both to susceptibility to dental caries, and to reactivity of tooth substance to various therapeutic chemical agents. In addition, it was early found that the information on age changes had a direct and useful application in the problems of age estimation that arise in anthropology and forensic medicine. The present project on age estimation has been in progress for some seven years, and involves both the validation and extension of proposed systems and exploration of new methods. The majority of the work has been directed toward an investigation of the most popular system, proposed by Gustafson (from Sweden), in which six histological features, known to change with age are assessed, and the resulting mathematical score is related to age on the basis of a regression line prepared from similar analysis of a tooth sample of

known ages. This method is being evaluated both for accuracy, and for value in comparison to ordinary gross visual estimation based strictly on experience. Further study is in progress on a technique which takes into account the age changes in structure of the outer enamel surface which have been found to progress with remarkable regularity. Numerous investigators have participated in this project, and Professor Miles was brought in because of his wide experience along the same line in England.

Methods Employed:

The different features of teeth which change with age are studied by gross examination, by microscopic study of longitudinal ground sections, and by examination of replicas of the outer enamel surfaces. Depending on the method of age determination involved, various scoring systems are used with samples of known age, and the appropriate statistical treatments applied for the derivation of standard age estimation curves. Specimens of ages known in the Laboratory but not revealed to the investigators are then assessed and age estimations are made by means of the standard curves. Accuracy of estimations is finally determined statistically.

Major Findings:

All of the tests thus far made indicate that the Gustafson method yields results somewhat less accurate than proposed when the technique was published. In the work to date, which has involved over 600 teeth and nearly 4000 ground sections, the approximate level of accuracy established indicates that roughly half the teeth can be aged within a five year error, and two thirds within ten. By the ordinary educated guess method, the results are almost identical for experienced observers. Since the Gustafson method has the advantage of objectiveness, work is now under way to determine if the accuracy level for inexperienced observers, who score much more poorly in visual guesses, can be improved through training in the measurement system. Other aspects of the project nearing completion include improvement of the Gustafson criteria, measurement system, and statistical treatment of the data. Considerable promise has been shown in preliminary tests of a system of age estimation through assessment of surface detail (by means of replicas examined microscopically) and these experiments will be continued.

Significance to Dental Research:

There is considerable demand from both anthropologists and medico-legal authorities for aid in age estimation. The available anthropological methods yield results which are subject to error of considerable magnitude, and the added information which can be provided from dental assessment is of obvious value. Further, when only fragmentary remains are available, the dental approach becomes extremely important, and is often the only one possible. In fact, most of the material received in this Laboratory falls in the latter category.

Part B. included: No

Serial No. NIDR-6
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Electron Microscopic Studies of the Ultrastructure of Developing and Mature Bone and Dentin.

Principal Investigator: Dr. S. Takuma

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives:

Observation of (1) normal osteogenesis of endochondral bone (2) early mineralization of dentin, and (3) tubular structure of mature dentin.

Methods Employed:

For studies of osteogenesis and dentinogenesis embryonic bone and tooth is fixed, embedded and sectioned for electron microscopy. In addition to microscopic examination, the presence and identification of crystalline material which may be present is investigated by means of electron diffraction. The tubular structure of mature dentin is studied by means of replicas made from the surfaces of cleaved fragments or ground sections which have been subjected to various chemical treatments.

Major Findings:

The developmental sequence in formation of endochondral bone has been studied under the electron microscope. Among the new data derived are the observations that (1) the earliest degenerative changes in the chondrocytes take place in the cytoplasmic organelles, (2) the matrix fibrils in cartilage occur in a fine network and not in bundles, (3) these fibrils show periodic banding at 220Å intervals, in contrast to the 640Å spacings observed in mature bone matrix, and (4) as in dentin, mineralization is preceded by incorporation of amorphous organic material, probably mainly mucopolysaccharide, within the fibrillar matrix.

A beginning has been made in the investigation of mineral deposition in the dentinal matrix. An idea has been gained of the sizes and shapes of the crystals first laid down, and the pattern in which they accumulate.

Previous work in this and other Laboratories has indicated that the area between the odontoblastic process and the wall of the dentinal tubule may be highly calcified. Prior to these observations it was variously thought that the intratubular space, which optically appears transparent, is real and filled only with organic material or fluid, or the space is unreal and due to post mortem specimen damage or optical illusion. In the present study, in which replicas and sections were made from fully mature dentin, the region in question has continued to appear highly calcified. The intratubular crystalline material can be isolated by acid etching or removed totally by decalcification, and the organic framework can be recovered through decalcification or removed by treatment with organic solvents.

Significance to Dental Research:

This study is essential to a more detailed understanding of the mechanisms of development and calcification of bone and dentin.

Proposed Course of Project:

Since Dr. Takuma has returned to Japan at the termination of his appointment as a Visiting Scientist, the work on bone

is being discontinued for the present. Certain aspects of the study of dentin calcification are being incorporated into other projects within this Laboratory.

Part B. included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Takuma, S. Electron Microscopy of the Developing Cartilagenous Epiphysis (accepted for publication, Arch. Oral Biol.)

Two other manuscripts recently submitted for publication

Honors and Awards relating to this project:

None

Serial No. NIDR-7
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Experimental Animal Caries

Principal Investigator: Dr. P. H. Keyes

Other Investigators: None

Cooperating Units: Dr. R. J. Fitzgerald, Gnotobiotic Section,
Laboratory of Microbiology (NIDR).

Man Years (calendar year 1959):
Total: 3 1/4
Professional: 1
Other: 2 1/4

Project Description:

Objectives:

This project has been a continuation of NIDR-8 (1958) in which various factors related to the induction of experimental caries in hamsters and rats have been studied. Resistance to caries in hamsters was previously thought to be due to constitutional and hereditary factors. Since the discovery that low caries activity, so called "resistance", is partly due to the absence of the necessary microflora, the major effort has been to investigate microbial facets of the problem in collaboration with Dr. Robert J. Fitzgerald of the Gnotobiotic Section, NIDR. Specifically, the objectives are:

1. To further study the transmissible nature of experimental caries using streptomycin-resistant and erythromycin resistant microorganisms as tracers.

2. To continue preliminary studies of the possible genetic basis for the difference in "susceptibility" between Osborne-Mendel and Black rats.

Methods Employed:

1. Pure cultures of microorganisms produced by the Gnotobiotic Section have been used to infect hamsters, rather than the fecal material originally employed.

   Hamsters have been placed on experimental testing procedures similar to those usually employed in this Laboratory. By using a group of animals which are not infected with the cariogenic flora, it has been possible to inoculate with pure cultures of organisms such as streptococci, lactobacilli, diphtheroids, etc. The infectivity and pathogenicity of various strains of streptococci made resistant to streptomycin have also been studied. These latter strains have been given to "non-infected" hamsters and caries activity thereby induced has been compared with that of naturally infected animals and with non-infected animals inoculated with regular streptococcal strains.

2. A more refined technic for assessing various compounds with a possible cariostatic potential has been achieved by using naturally infected weanling hamsters, 20 to 21 days old. These animals are fed the usual caries-test diet for two to three weeks before the test compound is introduced. During this short period active caries develops in the second and to some extent in the first molars. At this time, before the third molar has erupted, the "cariostatic" agent is introduced. By observing the progress of the disease in the live animal, it is possible to determine if the agent under test causes an arrestation of caries in the first and second molars and prevents the initiation and development of caries in the third molars.

3. By the use of the labelled organisms mentioned above preliminary studies have been undertaken to demonstrate further the transmissible nature of the cariogenic flora between animals caged together and from female to litter.

4. The incidence of caries has been studied in Osborne-Mendel and NIH Black rats which have been caged together and which have been bred together to determine if evidence of hereditary factors can be demonstrated with sufficient consistency and clearness to continue further studies along these lines.

Major Findings:

The colleborative study with Dr. Fitzgerald has yielded the following findings:

1. The inoculation of strains of streptococci isolated from a carious lesion in a hamster has been followed by the development of active caries in animals which otherwise would not have had the disease. Lactobacilli, diphtheroids, and several other types of organisms have not shown a pathogenic potential. By the use of streptomycin-resistant organisms, it has been possible to inoculate, to induce caries, and to recover and identify the inoculated strains. Thus for the first time under routine laboratory conditions Koch's postulates have been fulfilled, and specific bacteria have been demonstrated as etiologic agents in experimental caries. These modified organisms, however, do not appear to be so pathogenic as the original strains, and the experimental period must be considerably increased.

2. When cariostatic agents such as erythromycin are bioassayed by the methods previously described, caries is rapidly arrested in first and second molars and prevented from initiation in the third molars. Sodium chloride was not cariostatic, as was expected; and in the few tests made so far dibasic sodium phosphate has not been conclusively effective either in arrestation or in the prevention of caries initiation. Sodium fluoride (50 ppm) prevents the initiation of lesions in newly erupted teeth, but its effectiveness in arrestation has not been fully assessed todate.

3. Streptomycin-resistant streptococci have been isolated from weanlings born of mothers infected with these organisms, and also from animals placed in cages with the inoculated animals. This method of testing proves conclusively the transmissible nature of this flora.

4. When an Osborne-Mendel female is mated at the same time to an OM male and to a Black mate, her litter consists of part white and part black animals. Caries seems to be less active in hybrid young which carry the Black genes, than to the homozygous young which are white.

Significance to Dental Research:

Interest in the hamster as an animal for the study of dental caries seems to be reviving now that more is known about the nature of the disease. Infected animals have been sent to several other laboratories to permit investigators to establish colonies of their own. The use of pure culture of known cariogenic pathogenicity will be of use in many studies. The demonstration of Koch's postulates and the use of labelled microorganisms marks a real advance for future work. The rapid testing of cariostatic agents is now possible under conditions that can be closely observed. If the hereditary factors are responsible for the difference in caries activity between CAl and Black rats, it will be of interest to identify the mechanism involved.

Proposed Course of Project:

Inoculation with other strains and different types of organisms will continue under the collaborative program with the Gnotobiotic Section. The transmissible nature of the infection and various host-parasite relationships will be investigated further. The innovation of the use of labelled microorganisms as bacteriological tracers will permit the effect of various therapeutic procedures to be followed bacteriologically as well as pathologically. Additional work with rats will be undertaken to establish firmly the validity of the hypothesis that hereditary factors account for the difference in caries activity now observed.

Part B. Included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Keyes, P.H. The Infectious and Transmissible Nature of Experimental Dental Caries. Findings and Implications. (In press, Archives of Oral Biology).

Honors and Awards relating to this project:

Essay Contest of Chicago District Dental Society. $500.00 prize with Dr. Robert J. Fitzgerald (first author). Paper to be published in the J.A.D.A. 1960, entitled: "The Demonstration of the Etiologic Role of Streptococci in Experimental Caries in the Hamster".

Serial No. NIDR-8
1. Histology & Pathology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Diagnostic Oral and Surgical Pathology Utilizing Histochemical Techniques with Emphasis upon Periodental Disease and Neoplasia.

Principal Investigator: Dr. M. S. Burstone

Other Investigators: None

Cooperating Units: Dr. G. Glenner (NIAMD-66301-26), Dr. Paul N. Baer (NIDR), Dr. Louis Thomas (NCI), Dr. Robert Love (NCI-528), Dr. Gordon Tompkins (NIAMD-66301-20)

Man Years (calendar Year 1959):
Total: 1 1/4
Professional: 1/2
Other: 3/4

Project Description:

Objectives:

1. The application of existing and newly developed techniques to the study of freshly removed human surgical and other material, with emphasis upon oral tissues.

2. This type of study reveals tissue changes which are not visible with routine stains, and may contribute to the diagnosis of tumors as well as to the elucidation of the pathogenesis of neoplasia.

Methods Employed:

During the past five years a systematic histochemical study has been made in this laboratory of most human neoplasms removed in the Clinical Center. To date, over 400 surgical specimens have been frozen and dried for special study.

The specimens are obtained from patients scheduled for general and dental surgery, and also from selected cases admitted to the Clinical Center for special study. The specific procedures lean heavily on enzyme techniques which are considered in part in project report NIDR-6.

Major Findings:

1. In the previous report (1959) mention was made of studies on aminopeptidase in the stroma of tumors. This histochemical finding was first reported by the principal investigator and subsequently confirmed in foreign laboratories. During the last year Drs. Glenner (NIAMD), Burstone, and Meyer (NIAMD) have reported on what is considered to be the most extensive histochemical study ever made of human neoplasms. Most of the material consisted of frozen-dried tissues processed by the principal investigator. The work, reported in a recent issue of the J.N.C.I. describes hitherto unknown sites of proteolytic activity in human neoplasms.

2. Drs. P. N. Beer and M. S. Burstone have reported on what is considered to be the first enzyme histochemical study of deposit (calculus) formation on teeth. Deposits formed on films on human teeth were studied using histochemical techniques developed by the principal investigator. High esterase activity was correlated with deposit formation. This may elucidate a mechanism of calculus formation. In addition, esterase activity was visualized for the first time in oral bacteria.

Significance to Dental Research:

The application of histochemical techniques to investigations of neoplastic tissues have provided new information about enzymatic and other changes associated with pathological processes. Of particular interest are enzymatic characteristics of hard tissues (bone) undergoing resorption or apposition in periodontal disease. Moreover, the development of standardized methods enables histochemical techniques to be utilized in other disciplines.

Proposed Course of Project:

It would be desirable to expand this program so as to broaden the scope of the histochemical approach, and to utilize more fully these new techniques and concepts in the study of periodontal disease. In addition, further emphasis will be placed upon special staining procedures for diagnosis of oral abnormalities.

Part B. included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Savchuck, W. and Burstone, M.S. Calcification and Esterase Localization in Developing Dentin. J. Dent. Res. 37:1164-1166 (Nov.-Dec. 1958).

Burstone, M.S. Histochemical Demonstration of Acid Phosphatase Activity in Osteoclasts. J. Histochem. & Cytochem. 7:39-41 (Jan. 1959).

Burstone, M.S. and Fleming, T.J. A New Technique for the Histochemical Study of Smears. J. Histochem. & Cytochem. 7:203 (May 1959).

Glenner, G.G., Burstone, M.S. and Meyer, D.B. A Study of Aminopeptidase Activity in the Stroma of Neoplastic Tissue, With a Comparison of Histochemical Techniques. J. Nat. Cancer Inst. 23:857-872, 1959.

Baer, P.N. and Burstone, M.S. Esterase Activity Associated with Formation of Deposits on Teeth. Oral Surg., Oral Med. and Oral Path. 12:1147-1152 (Sept. 1959).

Honors and Awards relating to this project:

None

Serial No. NIDR-9
1. Histology & Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development and Application of Histochemical Methods in the Study of Hard and Soft Tissues.

Principal Investigator: Dr. M. S. Burstone

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1 1/4
Professional: 1/2
Other: 3/4

Project Description:

Objectives:

To develop new histochemical procedures and to modify existing ones for histopathological and histological studies.

Methods Employed:

Particular emphasis has been given to the microscopic demonstration of enzyme systems which may be implicated for example in the processes of bone apposition and resorption. The enzyme procedures which have been and are being developed include techniques for the demonstration of esterases, phosphatases, sulfatase, glucuronidase, dehydrogenases, and cytochrome oxidase. Entirely new substrates have been synthesized for these techniques. For the most part, the substrates consist of esters which release potentially chromogenic substances upon enzymatic hydrolysis.

Entirely new enzyme fluorescence techniques are being developed. In addition, new techniques for the demonstration of cytochrome oxidase enable the dye to be complexed with heavy metals, and

thus increase the sensitivity of the technique. Whenever feasible frozen-dried tissues are employed in order to enhance the quality of histological localization.

Major Findings:

1. A new finding, namely the presence of high succinic dehydrogenase activity in osteoclasts associated with active bone resorption has been intensively studied. In the previous project report, high acid phosphatase was observed in osteoclasts. It is felt that the aforementioned cells may be characterized by a distinct "enzyme spectrum".

2. Alkaline phosphatase activity has been revealed for the first time by fluorescence microscopy. This represents the first fluorescence enzyme technique ever to have been devised. It is known that fluorescence methods (e.g., the fluorescent antibody technique) are extremely sensitive. By analogy the newly developed fluorescent enzyme method is quite sensitive, and is capable of revealing sites of very low activity.

3. The new cytochrome oxidase technique has made it possible for the first time to visualize with the microscope oxidase activity of mitochondria of the human heart as well as other tissues.

Significance to Dental Research:

Histochemical techniques reveal biochemical characteristics of normal and abnormal hard and soft tissues. This represents a more accurate reflection of physiological and pathological processes as contrasted with interpretations based upon nonspecific staining reactions.

Proposed Course of Project:

Present investigations will be continued and extended. Included are studies on the effects of endocrines upon oral tissue enzyme systems.

Part B. included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Burstone, M.S. Acid Phosphatase Activity of Calcifying Bone and Dentin Matrices. J. Histochem. Cytochem. 7:147-148, March, 1959.

Burstone, M.S. New Histochemical Techniques for the Demonstration of Tissue Oxidase (Cytochrome Oxidase). J. Histochem. Cytochem. 7:112-122, March, 1959.

Honors and Awards relating to this project:

None

Serial No. NIDR-10
1. Histology & Pathology
3. Bethesda, Md

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Histochemical Studies of Connective Tissues.

Principal Investigator: Dr. H. M. Fullmer

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	3½
Professional:	1
Other:	2½

Project Description:

Objectives:

To determine the chemical composition and the reactive groups on normal and diseased connective tissues through the use of histochemical methods.

Methods Employed:

Human and animal tissues are obtained from autopsy and surgical material. Specimens to be sectioned are embedded either after freeze drying or after chemical fixation and dehydration. Under controlled conditions, the sections are exposed to various reagents designed to determine the site and quantity of a reactive group or substance.

Major Findings:

1. In 1958 a new stain, the peracetic-aldehyde fuchsin-Halmi stain, was developed which led to the discovery of a new connective tissue fiber. This has been designated the oxytalan fiber and is located principally in areas of stress such as the periodontal membranes, ligaments, tendons, the adventitia of blood vessels, epineurium and perineurium, and the connective tissue sheaths surrounding the hair follicles and sebaceous glands of the skin. To date, the fibers have been found in man and in several animals. The fibers have protein and mucopolysaccharide components. During the course of studies of the embryological development of the fibers, it was found that their development was associated with

Part [illegible] (Continued)

mucopolysaccharide production and maturation. The histochemical reactions of oxytalan fibers appear to indicate a relation to elastic fibers.

2. The development of another new stain, the peracetic-orcein-Halmi stain, has resulted in the demonstration of an elastic-like component of mucous connective tissues. It is believed that the substance reactive with the peracetic-orcein-Halmi stain is sometimes the same as that reactive with the peracetic-aldehyde fuchsin-Halmi stain However, the peracetic-orcein-Halmi stain is more restricted in its reactivity; i.e., it reacts with fewer types of mucopolysaccharides, and, as a result, demonstrates a previously undescribed fibril in mucous connective tissues (in the pulps of teeth, in umbilical cord and in the rooster comb). The development and use of the two stains described above has aided considerably in the development of a unified concept of connective tissues. This has permitted the observation that tendon and mucous connective tissue cells, just as connective tissue cells generally, produce an elastic-like component, and that the nature of this product varies with the anatomic site and function.

A relation during development between one connective tissue product (mucopolysaccharide) and another connective tissue product (elastic-like fibers) was also demonstrated.

3. Oxytalan fibers were observed to develop in dental granulomas and radicular cysts. This is interesting since it demonstrates the potential of connective tissue cells in the periodontium to produce oxytalan fibers (a) in an adult and (b) under pathological conditions.

4. Since a peracetic acid oxidation was required to demonstrate oxytalan fibers, and since the data indicated that the stainable component was probably a mucopolysaccharide, an investigation of the effects of peracetic acid on mucopolysaccharides was undertaken. It was found for the first time that:

(a) β-glucuronidase specifically digests certain of the mucins if they are preoxidized with peracetic acid. The periodic acid-Schiff stain is reversed in these preparations.

(b) peracetic acid oxidation of tissue sections also accelerates digestion of certain mucopolysaccharides by some proteolytic enzymes.

Part A (continued)

(c) The peracetic acid-aldehyde fuchsin-Halmi stain demonstrates a mucopolysaccharide heretofore not observed histochemically. Its chemical identity is unknown.

(d) The peracetic acid-aldehyde fuchsin-Halmi, and the Hale stain demonstrate a mucopolysaccharide in mast cells that does not react metachromatically with basic stains.

(e) Umbilical cord and developing tooth pulps contain a mucopolysaccharide reactive with the peracetic acid-aldehyde fuchsin-Halmi stain that does not induce metachromasia with basic dyes.

The mechanism whereby peracetic acid induces staining of mucopolysaccharides by aldehyde fuchsin, and alters mucopolysaccharides so that they are susceptible to various mucopolysaccharases and some proteolytic enzymes is unknown.

5. In collaboration with investigators in the NHI, an interesting experiment on the mechanism of enzymatic action was conducted. A mirror image of ascorbic acid (D-ascorbic acid) was prepared and administered in appropriate dosage to scorbutic guinea pigs. Contrary to two previous reports, it was demonstrated that D-ascorbic acid does have partial vitamin C activity, i.e., it allows (a) survival of the animals, (b) weight maintenance of the animals, and (c) dentinogenesis - although possibly imperfect as demonstrated by histochemical stains, but it does not prevent the characteristic hemorrhages of scurvy.

6. While using a section of abdominal skin from a patient who died of amyotrophic lateral sclerosis as a control, it was discovered that it reacted differently than normal when stained with the peracetic acid-aldehyde fuchsin-Halmi stain. The cooperation of investigators in the NINDB was sought and subsequently dermal biopsies from 46 patients with amyotrophic lateral sclerosis have been reported. As a result, it was demonstrated for the first time that a characteristic connective tissue disorder exists in at least 60% of patients with ALS. The disorder is characterized by (a) a distinctive elastosis, (b) an increase of mucopolysaccharide, (c) a degeneration and elastosis of arrector pili muscles, (d) focal areas of apparently regenerated connective tissues, and (e) several characteristic alterations of collagen. These changes were found in a number of patients from the United States and from Guam. Individuals severely afflicted with ALS manifest the disorder more frequently than those less severely affected.

Part A (continued)

Since the skin specimens from several patients having various neurological disorders resulting in loss of motor innervation to the biopsied site did not manifest the changes ascribed to ALS, a primary disorder of connective tissue in this disease is suggested rather than being secondary to a loss of motor innervation. The characteristic changes of connective tissues found in ALS are such that it is doubtful that they would have been observed without the use of the peracetic acid-aldehyde fuchsin-Halmi stain.

Significance to Dental Research:

Periodontitis and periodontosis are diseases of connective tissues. It is believed that information gained about the nature of normal connective tissues and their alterations in the diseased states will contribute to a better basic understanding of the pathological processes involved in periodontal disease. The ultimate importance of the discovery of a new connective tissue fiber in the periodontal tissues, and in other tissues subjected to stress, is still unknown. However, there is some indication (not yet fully demonstrated) that oxytalan fibers are more resistant than collagen to destruction by the lesion of periodontitis.

Since a considerable percentage of patients with amyotrophic lateral sclerosis have a connective tissue disorder that on the basis of preliminary studies appears to be a primary part of the disease, a new concept of the nature of this disease must be developed.

The finding that vitamin C may have multiple actions is significant to all interested in cell biology.

Proposed Course of Project:

Continued attention will be given to the histochemical study of normal and diseased connective tissues and their alterations during development and with age. Time will also be devoted to the further exploration of the chemical composition function and distribution of the newly found fibers.

Histochemical tests of phosphorylations will be conducted on skin specimens of patients afflicted with ALS.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Fullmer, Harold M. The Peracetic-Orcein-Halmi Stain: A stain for connective tissues. Stain Technol. 34: 81-84, March 1959.

Fullmer, Harold M. Observations on the Development of Oxytalan Fibers in the Periodontium of Man. J. Dent. Res., 38: 510-518, May-June, 1959.

Burns, J. J., Fullmer, Harold M., and Dayton, Peter. Observations on the Vitamin C activity of D-Ascorbic Acid. Proc. Soc. Exper. Biol. & Med. 101: 46-49, 1959.

Fullmer, H. M. Differences in mechanism in staining reactions for mast cells. Nature, 183: 1274-1275, May 2, 1959.

Fullmer, H. M. Effect of Peracetic acid on the Enzymatic Digestion of Mucopolysaccharides: Reversal of the PAS Staining Reaction of Mucin. J. Histochem. & Cytochem. (In press)

Fullmer, H. M. Observations on the Development of Oxytalan Fibers in Radicular Cysts and Dental Granulomas. A.M.A. Arch. Path. (In press)

Fullmer, H. M., Siedler, H., Krooth, R., Kurland, L. A. Connective Tissue Disorder in Amyotrophic Lateral Sclerosis. Neurology (In press).

Honors and Awards Relating to this Project:

None

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1959

MICROBIOLOGY:

11. R. J. Fitzgerald and H. V. Jordan: The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease.

12. E. G. Hampp and S. E. Mergenhagen: Studies on Experimentally Induced Fusospirochetal Infection in Rabbits.

13. A. Howell, Jr. and L. Pine: A Study of Oral Filamentous Organisms and Related Bacteria.

14. A. Howell, Jr. and A. A. Rizzo: The Relationship of Pathogenic Fungi to Diseases of the Oral Cavity. I. Incidence and Identity.

15. H. V. Jordan and R. J. Fitzgerald: Inhibitors and Experimental Dental Caries.

16. S. E. Mergenhagen and E. G. Hampp: Endotoxins from Oral Bacteria.

17. T. A. Nevin: Studies of Interactions Between Oral Organisms.

18. T. A. Nevin: Bacteremias Following Exodontia and Periodontal Treatment.

19. R. R. Omata: Studies on the Physiology of Oral Fusobacteria and on Proteolytic Enzymes of Microbial Origin.

20. A. A. Rizzo: Methods of Using the Hamster Cheek Pouch for Periodontal Research.

21. A. A. Rizzo: A Comparison of the Pathology of Chronic Magnesium Deficiency and of Chronic Dilantin Sodium Toxicity in Rats.

22. M. Rogosa: Immunological, Biochemical and Pathological Studies of Oral Bacteria.

23. H. W. Scherp: Manifest and Inapparent Viral Infections of Oral Tissues.

24. H. W. Scherp and S. E. Mergenhagen: Digestion of Native and Altered Collagens by Oral Microorganisms in Relation to Periodontal Disease.

25. T. Shiota: Nutrition and Metabolism of Lactic Acid Bacteria.

Serial No. NIDR-11
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Use of Germfree and Gnotobiotic Animals in the Study of Problems Relating to Oral Disease

Principal Investigators: Dr. R. J. Fitzgerald
Dr. H. V. Jordan

Other Investigators: Dr. P. H. Keyes
Dr. H. R. Stanley
Dr. E. G. Hampp

Cooperating Units: Germfree Unit, Sanitary Engineering Branch,
Division of Research Services (DRS 6.5)
Lobund Institute, University of Notre Dame

Man Years (calendar year 1959):

Total:	3 3/4
Professional:	1
Other:	2 3/4

Project Description

Objectives:

To study caries, dental calculus formation and periodontal disease in germfree and gnotobiotic animals.

Methods Employed:

1. The standard procedures and equipment common to all studies involving germfree animals and gnotobiotic animals (i.e., germfree animals purposely infected with known types of microorganisms) are employed.

2. In addition, a technique has been adapted by which strains of bacteria may be labelled and their distribution in the conventional experimental animal determined. This permits the use of conventional animals for certain studies formerly possible only in gnotobiotic animals.

Part A (continued)

Major Findings:

1. The occurrence, chemical analysis, and electron and X-ray diffraction patterns of the calcareous deposits on the molar teeth of conventional rats have been studied. This material was found to be hydroxyapatite which is the same substance found in most human dental calculus. Its formation in these experimental animals was apparently not inhibited by incorporation of the antibiotics chloramphenicol or chlortetracycline in the animals' diet.

2. Similar deposits occurring on the teeth of germfree rats have also been found to contain hydroxyapatite by means of X-ray diffraction studies. These results indicate that microorganisms are not, as generally considered, essential for dental calculus formation. However, differences in the frequency and amount of calculus observed in the germfree and the conventional animal suggest that microorganisms may contribute to the severity of the calculus deposit.

3. In collaboration with Dr. Paul Keyes, it has been proved that certain strains of streptococci, isolated from a carious lesion in the hamster, can cause dental caries when inoculated in a strain of hamsters previously thought to be caries resistant. This observation has been confirmed through the use of the streptococci "labelled" by inducing resistance to the antibiotic, streptomycin. These labelled organisms could be implanted in the oral cavity of "caries inactive" hamsters, which subsequently developed dental caries, whereas uninfected animals of the same strain did not do so. The labelled organisms could be recovered and distinguished from the many other types present in the animal by culturing them on media containing sufficient amounts of streptomycin to prevent the growth of all but the labelled microorganisms. By this means these microorganisms could be recovered from the mouth, carious lesions and the feces of infected hamsters. These organisms, and caries activity, could be passed from infected to uninfected hamsters by means of the feces of the infected animals. The labelled streptococci subsequently appeared in the offspring of infected females for several generations and these offspring are now caries active.

Part A (continued)

4. Streptococci isolated from the feces or oral cavity of caries-inactive hamsters do not produce caries in this test system nor do lactobacilli or diphtheroid organisms isolated from the oral cavity of caries-active hamsters. A rat strain of streptococcus which will induce caries in germfree rats was ineffective in the hamster although it could be shown to persist in the hamster oral cavity. These findings illustrate the remarkable degree of host and site specificity of microorganisms in the caries process.

Significance to Dental Research:

Through the use of germfree animals, problems involving the etiology of oral lesions and conditions may be studied in the absence of the complex oral flora which has hindered previous investigations. The use of antibiotic-labelled microorganisms now makes it possible to perform certain of these studies in conventional experimental animals in the normal mixed microbial environment of the oral tissues, i.e., under conditions more closely approximating those found in human oral infections.

The observation that deposition dental calculus can occur in the germfree animal indicates that our concepts regarding the etiology of this process must be broadened to include more active consideration of the role of other host factors as well as the oral microbial flora.

Proposed Course of Project:

Future studies will be concerned with the exploitation of observations discussed in this report, and will attempt to discover the mechanisms by which the active strains of streptococci produce caries and the factors contributing to calculus formation.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Fitzgerald, R. J., Parramore, M. L., and MacKintosh, M. E. Antibiotic Sensitivity of Oral Strains of Veillonella. Antib. and Chemother. 9:145-150, 1959.

Jordan, H. V., Fitzgerald, R. J., and Faber, J. E. A Survey of Lactobacilli including Pigmented Strains from the Oral Cavity of the White Rat. J. Dental Res. 36:611-617, 1959.

Hampp, E. G., and Fitzgerald, R. J. The In Vitro Effects of Antibiotics on Oral Spirochetes. J. Dental Res. 38: 947-952, 1959.

Fitzgerald, R. J.; Jordan, H. V., and Stanley, H. R. Dental Caries and Gingival Pathology in the Gnotobiotic Rat. J. Dental Res., in press.

Fitzgerald, R. J., Jordan, H. V., Scott, D. B., and McCann, H. G. Dental Calculus and Antibiotics in the Rat. J. Dental Res., in press.

Jordan, H. V., Fitzgerald, R. J., and Bowler, A. E. Inhibition of Experimental Caries by Sodium Metabisulfite and its Effect in the Growth and Metabolism of Selected Bacteria. J. Dental Res., in press.

Honors and Awards Relating to this Project:

None

Serial No. NIDR-17
1. Microbiology
3. Bethesda, Md

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on Experimentally Induced Fusospirochetal Infection in Rabbits.

Principal Investigators: Dr. E. G. Hampp
Dr. S. E. Mergenhagen

Other Investigators: Dr. R. R. Omata

Cooperating Units: American Dental Association

Man Years (calendar year 1959):
Total: 1 1/4
Professional: 1
Other: 1/4

Project Description:

Objectives:

1. To investigate the infectivity of pure strains of the oral spirochetes and fusobacteria, both separately and in combination, for rabbits by intracutaneous inoculation.

2. To determine the mechanisms of synergistic infections elicited by the spirochetes and fusobacteria; in particular, (a) anachoresis of fusobacteria by spirochetal lesions, and (b) enhancement of the experimental lesions by bacterial mucopolysaccharases.

3. To study the intracutaneous lesions initiated in rabbits by an anaerobic oral spirillum, Selenomonas sputigena.

4. To relate these experimental infections to human Vincent's infection.

Part A (continued)

Methods Employed:

1. Intracutaneous injection of rabbits with pure strains and various combinations of the small oral treponemes, Borrelia vincentii, Borrelia buccalis, Fusobacterium nucleatum, and Fusobacterium polymorphum.

2. Dark-field microscopy and cultural examinations of the various animal lesions to determine the survival time of individual organisms employed.

3. Gross examination of abscesses at periodic intervals to determine changes in size, extent, differences, and duration of lesions.

4. Histologic examination of tissue sections for determination of progress, cell response and any differences that may be observed between the various lesions produced during these experiments.

5. Silver staining of tissue sections and use of other bacterial stains for determining the locations and phagocytosis of spirochetes, fusobacteria, and spirilla.

6. Use of heat-treated and nonheat-treated bacterial cells, filtered culture supernatants and sonic oscillated cells to investigate the mechanism of infection in animals.

Major Findings:

1. During this period studies were conducted on the elicitation of reproducible intracutaneous lesions with pure individual strains of a small oral treponeme, Borrelia vincentii and Borrelia buccalis. These infections resulted in abscesses which persisted up to one month but from which the various spirochetes could be recovered only up to four days. Sonically oscillated and heat-killed cells potentiated similar gross lesions on intracutaneous inoculation; however, they differed somewhat from a histologic point of view although not to any great extent. A toxic factor is apparently contained in the microorganisms and not in the culture supernatants or filtrates and is presumably an endotoxin. Studies are in progress on this phase of the problem.

Part A (continued)

2. In addition, these studies were extended to include *Fusobacterium nucleatum* and *Fusobacterium polymorphum*, both separately and in combination with the small oral treponemes and *B. vincentii*. Intracutaneous abscesses were routinely produced in rabbits with the fusobacteria alone, and they have also existed up to one month. Similar lesions were elicited with heat-killed cells. Exotoxic activity was not demonstrated in the supernatant culture fluids of these microorganisms. When a given strain of fusobacteria and spirochetes were cultured together, or grown separately and recombined, the lesions subsequently produced by these synergistic combinations were more fulminating and grossly larger than when either organism was used alone. This was particularly evident in the former instance. Microscopically and culturally, it was found that the fusobacteria invaded from their individual lesions to spirochete lesions in their proximity on the same side of the animal. Further, an anachoretic effect was elicited when fusobacteria were introduced intravenously 24-48 hours following the initiation of intradermal spirochete abscesses and the localization of the fusobacteria in these lesions produced a synergistic effect. This problem is under active investigation.

3. Further, it was found in preliminary animal studies that the anaerobic oral spirillum, *Selenomonas sputigena*, when injected intracutaneously in rabbits, elicited large spreading lesions with areas of abscess formation. From the point of inoculation on the shaved back of the animals, the elevated, erythematous and edematous lesions spread downward to form triangular areas with broad bases at the nipple line. These lesions were more extensive and fulminating than any yet produced with any of the other oral bacteria. The histologic and bacteriologic information is not available at this time. Preliminary studies show that *S. sputigena* produces an endotoxin.

Significance to Dental Research:

1. This is the first instance, in the last 8 decades, that pure cultures of oral spirochetes and oral fusobacteria, both singly and in combination, were shown to

Part A (continued)

be capable of initiating experimental animal lesions. All evidence in the past on the pathogenicity of these organisms was presumptive in nature.

2. A new method is available which will afford the investigators an opportunity to study the etiologic significance of oral spirochetes and oral fusobacteria in Vincent's infection.

3. Since the oral spirochetes, as well as the fusobacteria, have been suggested as etiologic agents in periodontal disease by certain early workers in the field, these studies will afford an opportunity to further study this assumed relationship.

4. Studies with the oral spirillum may produce information concerning the initiation of periodontal disease.

Proposed Course of Project:

These investigations will be extended by the approaches previously described in order to elucidate the mechanisms of these experimental infections and ultimately to relate the findings to diseases of oral origin, in particular, to that group of oral ulcerative diseases commonly referred to as Vincent's infection.

Part B included   Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Hampp, E. G. Review of the Literature Pertaining to Oral Microbiology. J. Am. Dent. Assoc., **58**: 27-33, 1959.

Hampp, E. G., and Nevin, T. A. The Substitution of Known Compounds for Ascitic Fluid in the Cultivation of *Borrelia vincentii*. J. Bacteriol., **77**: 800-803, 1959.

Nevin, T. A., and Hampp, E. G. A Partially Defined Medium for the Cultivation of *Borrelia vincentii*. J. Bacteriol., **78**: 263-266, 1959.

Hampp, E. G., and Fitzgerald, R. J. The *in vitro* Effects of Antibiotics on oral spirochetes. J. Dent. Res., **38**: 947-950, 1959.

Hampp, E. G., Mergenhagen, S. E., and Omata, R. R. Studies on Mucopolysaccharase Activity of Oral Spirochetes. J. Dent. Res., 38: 979-982, 1959.

Rogosa, M., Hampp, E. G., Nevin, T. A., Wagner, H. J., Jr., Driscoll, E. J., and Baer, P. N. Blood Sampling and Cultural Studies in the Detection of Postoperative Bacteremias. J. Am. Dent. Assoc., in press.

Hampp, E. G., Scott, D. B., and Wyckoff, R. W. G. Morphologic Characteristics of the Oral Fusobacteria as Revealed by the Electron Microscope. J. Bacteriol., in press.

Serial No. [illegible]
2. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: A Study of Oral Filamentous Organisms and Related Bacteria.

Principal Investigators: Dr. A. Howell, Jr.
Dr. L. Pine

Other Investigators: Mr. W. C. Murphy, III

Cooperating Units: Duke University School of Medicine

Man Years (calendar year 1959):
Total: 1 3/4
Professional: 1 1/2
Other: 1/4

Project Description:

Objectives:

1. To study the physiological characteristics of organisms isolated from bovine actinomycosis and compare them with strains of Actinomyces isolated from humans.

2. To study the morphological and physiological characteristics of various types of filamentous organisms and related bacteria isolated from selected oral samples.

Methods Employed:

Standard bacteriological procedures, modified as necessary for the organisms under study, are being used. Organisms are being studied on the basis of (1) fermentation of 20 carbohydrates; (2) oxygen tolerance; (3) other biochemical tests; and (4) animal pathogenicity.

Part A (continued)

Major Findings:

1. Five strains of organisms, isolated in pure culture by Dr. Pine from bovine actinomycosis and 6 strains recently isolated from similar lesions by other investigators have been compared. They were found to comprise a homogeneous group and, on the basis of morphology, to represent a species of Actinomyces. It was concluded (a) that these organisms should be designated as Actinomyces bovis; and (b) that they are morphologically and physiologically distinct from strains of Actinomyces isolated from humans. The results of this investigation are now being incorporated into a manuscript for publication.

2. Detailed study of morphological and physiological characteristics of approximately 20 strains of filamentous organisms isolated from hamsters (Project NIDR-14) have shown them to be essentially aerobic; to require carbon dioxide for growth in certain media; to dissociate very readily; and to produce acid from 10 of 20 carbohydrates tested.

Significance to Dental Research:

The establishment of a clear-cut differentiation between the species of Actinomyces involved in human and bovine actinomycosis resolves an etiological problem that has hindered understanding of the epidemiology and pathogenesis of these diseases since their discovery 80 years ago.

A more complete study of the numerous filamentous organisms and related forms indigenous to the oral cavity should lead to easier identification of these bacteria and to a better understanding of their possible role in oral diseases.

Proposed Course of Project:

To continue the study of unidentified and unclassified filamentous organisms and related forms, including the diphtheroids, isolated from oral samples.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Howell, A., Jr., Murphy, W. C., Paul, F., and Stephan R. M. Oral Strains of *Actinomyces*. J. Bact., 78: 82-95, 1959.

Honors and Awards relating to this project:

None

Serial No. [illegible]
1. Microbiology
3. Bethesda, Md

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: The Relationship of Pathogenic Fungi to Diseases of the Oral Cavity. I. Incidence and Identity

Principal Investigators: Dr. A. Howell, Jr.
Dr. A. A. Rizzo

Other Investigator: Dr. R. M. Stephan

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2
Professional: 3/4
Other: 1 1/4

Project Description:

Objectives:

1. To study the incidence and identity of pathogenic or potentially pathogenic fungi in the "normal" mouth.

2. To study the possible relationship of these organisms to dental caries, calculus formation, and to other diseases of the oral cavity or related areas.

Methods Employed:

As in previous years, considerable emphasis has again been placed on the incidence and identity of both filamentous and yeast-like organisms in the oral cavity. For this purpose standard bacteriological methods for the isolation and identification of these organisms, modified for the organisms under study, have been employed.

Part A (continued)

1. Selected oral samples were obtained from various types of individuals (followed in the Clinical Center), including patients with caries, periodontal disease, stomatitis, etc. During the past year, in collaboration with Dr. Stephan, 138 oral samples (plaque material, scrapings from periodontal pockets, etc.) and 34 saliva samples from 25 patients have been studied. In addition, in collaboration with Dr. Rizzo, 26 specimens from 4 patients have been studied to determine the sequence of bacteriological changes, if any, during the formation of calculus. These have been taken at intervals varying from 2 days to over 2 months after complete scaling and cleaning of the areas from which the samples were subsequently taken.

2. In addition to the patient material listed above, a pilot study was made of the possible incidence of filamentous organisms in certain animals. For this purpose samples were taken from the gingival crevice of 11 golden and 12 albino hamsters and 1 cream hamster. These animals had been maintained on various diets by Dr. Paul Keyes and showed varying degrees of caries.

Major Findings:

1. Analysis of the studies of the 138 oral samples and 34 salivas confirms the conclusion, based on earlier work, that there are probably significant differences in the incidence of filamentous forms in different areas of the oral cavity and that, in general, they are much less frequent in saliva than in plaques or periodontal pockets. Large numbers of these organisms were found, in general, only in plaques from carious lesions and in certain types of periodontal pockets. Examination of these data, and those of previous studies in this series, on a patient basis, indicates that these organisms may be isolated from the majority of individuals studied. However, the relative incidence varies with the individual. For example, they were isolated in almost pure culture from dental plaques and carious lesions of most of the individuals studied who had rampant caries, and in similar large numbers in some instances from the gingival pockets of patients with periodontal disease.

With few exceptions, Candida albicans was the only yeast-like organism isolated from any of the oral samples. This was isolated only from plaques from carious lesions and from saliva of these same individuals.

2. Preliminary studies on the bacteria associated with the development of calculus indicate that a variety of microorganisms may be involved.

In early samples, gram-positive and gram-negative cocci (Streptococcus, Veillonella, and Neisseria) and Fusobacterium appear to be the predominant organisms but many other types of bacteria are present in small numbers. Filamentous organisms do not begin to appear until approximately the 10th to 14th day and do not become the predominant organism until much later. Even then, contrary to previous reports based on histological examinations, various bacteria may be isolated from specimens of calculus in addition to filamentous organisms. Chief among these are species of Fusobacterium, Streptococcus, Neisseria and Veillonella.

3. A filamentous organism, tentatively identified as a species of Nocardia, was isolated in almost pure culture from the gingival crevices of 9 of 11 golden hamsters sampled; 2 of 12 albino hamsters; and one cream hamster.

Significance to Dental Research:

1. The finding that species of Actinomyces may be the predominant organism in plaques from carious lesions in patients with rampant caries indicates that these organisms may play a major role in caries formation. Similarly, they may play a significant role, as reported by other investigators, in the formation of calculus and the production of periodontal disease. Further study is necessary to evaluate these tentative conclusions.

2. The isolation of a Nocardia-like organism from the gingival crevices of certain types of hamsters is an interesting finding but its significance remains to be determined.

Proposed Course of Project:

1. To continue the study of the bacteriology of calculus formation in collaboration with Dr. Rizzo, Laboratory of Microbiology, NIDR.

2. To continue the study of the incidence of filamentous organisms in the oral cavity of patients with rampant caries.

3. To continue the study of the incidence and possible significance of filamentous organisms in the oral cavity of the hamster.

Part B included No.

Serial No. NIDR-17
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Inhibitors and Experimental Dental Caries

Principal Investigators: Dr. H. V. Jordan
Dr. R. J. Fitzgerald

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2 1/4
Professional: 1
Other: 1 1/4

Project Description:

Objectives:

To test compounds which can be rationally expected to inhibit experimental caries in rats by inhibition of growth or metabolic activities of the oral microflora. This is a continuation of NIDR-15 (1958).

Methods Employed:

Present selection of compounds is based on their *in vitro* effects on the growth and glycolysis of certain oral bacteria. Growth inhibition of selected oral organisms by the compounds is tested by standard bacteriological procedures. The ratio of lactic acid produced to glucose utilized by certain lactic acid organisms is measured to give an indication of the effect on glycolysis. The compounds selected are then incorporated into a coarse corn particle, high sugar diet (NIDR 585) to test their effect on experimental caries. In certain cases the work on glycolysis is extended to a more complete carbon balance study.

Part B (continued)

Major Findings:

1. Addition of 0.9, 0.3, and 0.15 per cent sodium metabisulfite to diet 585 reduced occlusal fissure caries in rats by 77, 64, and 33 per cent respectively. This compound was also inhibitory to the *in vitro* growth of a wide variety of microorganisms including lactobacilli, streptococci, micrococci, candidae, leptotrichiae, diphtheroids, neisseriae, veillonellae, and gaffkyae. At subinhibitory concentration, the glucose metabolism of a streptococcus from the rat was shifted away from homofermentation resulting in a relative decrease in the ratio of lactic acid produced to glucose used. This strain has been shown to produce dental caries in gnotobiotic rats.

2. Observations on reduced lactic acid production under the conditions described above have led to a more complete investigation of the glycolysis of this organism based on carbon balance studies. When this streptococcus was grown in the presence of 0.005 per cent sodium metabisulfite, lactic acid production was reduced by about 20 per cent. Ethanol and CO_2 production were both increased 3-4 fold over the normal fermentation. There was also an increase in diacetyl, acetoin and 2,3-butanediol. Formation of acetic acid and formic acid did not appear to change significantly.

3. Since the physiological action of sodium metabisulfite is considered to be based on its capacity to bind carbonyl compounds with the formation of addition compounds, another chemical agent of this type has been tested. Dimedone (5,5-dimethyl-1, 3-cyclohexanedione) incorporated into diet 585 at 0.4 per cent gave a 55 per cent reduction in the caries score. At 0.4 per cent, the highest concentration tested, dimedone was only partially inhibitory to the growth of a variety of oral organisms. Effects of this compound on streptococcal glycolysis are now under investigation.

Significance to Dental Research:

The use of specific inhibitors to retard the initiation or development of experimental caries may lead to a better understanding of the physiological pathways leading up to the caries process. The studies described in this project report are concerned with compounds which have a known chemical effect, i.e., affinity for carbonyl groups, which results in a measurable physiological response of cariogenic microorganisms.

Part A continued:

Compounds such as these, with a dual inhibitory action, may be particularly interesting to test clinically as anticaries agents. At higher concentrations ($>0.1\%$) they are inhibitory to bacterial growth and at lower concentrations they alter metabolism in such a way that the bacteria produce less acid and hence may be less cariogenic. This latter effect, which may more closely approximate conditions in dental plaque, avoids the undesirable consequences resulting when the balance of the oral flora is upset by bactericidal agents.

Proposed Course of Project:

Further tests of these and other carbonyl-binding compounds are under way. Additional experiments on sodium metabisulfite are being conducted, particularly in regard to its effect on vitamins in the diet, and on the vitamin-producing flora of the intestine. Compounds containing other sulfur radicals are under test.

Studies on streptococcal glycolysis are being extended, particularly to those strains which have been shown by gnotobiotic experiments to be cariogenic. Comparison of the glucose metabolism of these active strains with other streptococcal strains demonstrated to be not cariogenic is contemplated.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Jordan, H. V., Fitzgerald, R. J., and Faber, J. E. A Survey of Lactobacilli including Pigmented Strains from the Oral Cavity of the White Rat. J. Dental Res. 38: 611-617, 1959

Fitzgerald, R. J., Jordan, H. V., and Stanley, H. R. Dental Caries and Gingival Pathology in the Gnotobiotic Rat. J. Dental Res., in press.

Fitzgerald, R. J., Jordan, H. V., Scott, D. B., and McCann, H. G. Dental Calculus and Antibiotics in the Rat. J. Dental Res., in press.

Jordan, H. V., Fitzgerald, R. J., and Bowler, A. E. Inhibition of Experimental Caries by Sodium Metabisulfite and its Effect on the Growth and Metabolism of Selected Bacteria. J. Dental Res., in press

Honors and Awards Relating to this Project:

None

Serial No. [illegible]
1. Microbiology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Endotoxins from Oral Bacteria

Principal Investigators: Dr. S. E. Mergenhagen
Dr. E. G. Hampp

Other Investigators: None

Cooperating Units: American Dental Association

Man Years (calendar year 1959):
Total: 1 1/4
Professional: 1/2
Other: 3/4

Project Description:

Objectives:

1. To determine whether members of the oral microbiota possess endotoxins which could be operative in the pathogenesis of periodontal disease.

2. To analyze the mechanism of action of these endotoxins on host tissue and to study the role they may play in experimental infections with oral bacteria.

Methods Employed:

Tryptic digestion according to the procedure of Raistrick and Topley has been applied to oral bacteria with a view to releasing soluble endotoxins from the cells. Cells have been fractionated also by the two-phase phenol-water system of Westphal. Tryptic digests and isolated fractions were tested for toxicity by intracutaneous injection in rabbits and intraperitoneal injection in mice. Development of lesions from the primary injection in rabbits was followed grossly and histologically. Similar observations were made on rabbits given a second injection intravenously to elicit the localized Shwartzman reaction. Toxicity for mice was calculated as the 50 per cent lethal dose.

Part A (continued)

Major Findings:

1. Tryptic digestion of strains of *Veillonella*, *Selenomonas*, and *Fusobacterium* released soluble endotoxins, as demonstrated by their production of inflammatory lesions with ischemic necrosis on intracutaneous injection in rabbits and by their ability to produce the dermal Shwartzman phenomenon. That these reactions could not be elicited by all bacteria indigenous to the oral cavity was shown by the negative results with tryptic digests of oral anaerobic streptococci and diphtheroid bacilli.

2. A lipopolysaccharide-containing endotoxic fraction, isolated by the phenol-water procedure from veillonella cells, has been characterized chemically as follows: it contained 10 per cent Kjeldahl nitrogen, 4 per cent protein, 4.2 per cent phosphorus; absorbed strongly at 260 mμ, indicating nucleic acid components; gave a strong Molisch test for carbohydrate; and yielded 5.6 per cent lipid after acid hydrolysis. The associated denatured protein fraction contained at least 90 per cent protein and gave a negative Molisch test. The LD_{50} of the lipopolysaccharide fraction was 1.0 mg. for Swiss Webster mice and 2.0 mg. for CAF-1 mice. As little as 30 μg. produced inflammatory necrotic lesions in the skin of rabbits and as little as 4 μg. prepared sites in rabbit skin for the local Shwartzman reaction. The size of the primary inflammatory lesion (24 hours) or the hemorrhagic necrosis of the Shwartzman reaction appeared to be proportional to the amount of the endotoxin employed. Comparable ranges of doses of the denatured protein fraction were not toxic upon intracutaneous injection in the rabbit and were not lethal to mice.

3. A similar highly toxic, water-soluble endotoxin has been isolated from a strain of *Selenomonas sputigena*.

Significance to Dental Research:

Toxic products derived from the cells of the bacteria indigenous to the gingival sulcus have long been suspected as possible factors in the tissue destruction seen in periodontal disease. The present data provide the first

Part B (continued)

direct evidence that pure cultures of certain of the oral bacteria commonly implicated in periodontal disease possess lipopolysaccharide endotoxins which have the biological and chemical characteristics of the well-known endotoxins derived from other gram-negative bacteria that have a high degree of pathogenicity. Investigations of the oral microbial sources of endotoxins contribute directly to our understanding of periodontal disease.

Proposed Course of Project:

To continue and expand the studies outlined above with particular emphasis on the role of endotoxins in experimental infections with the fusospirochetal complex.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Mergenhagen, S.E., Endotoxic Properties of Oral Bacteria as Revealed by the Local Shwartzman Reaction. J. Dent. Research, in press.

Honors and Awards relating to this project:

None

[illegible]
1. Microbiology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies of Interactions Between Oral Organisms

Principal Investigator: Dr. T. A. Nevin

Other Investigator: Dr. E. G. Hampp

Cooperating Units: American Dental Association

Man Years (calendar year 1959):
Total: 1 1/2
Professional: 3/4
Other: 3/4

Project Description:

Objectives:

1. To study the biochemical bases of the dynamic equilibria which determine the complex nature of the flora of the mouth.

2. To investigate the occurrence of, and the nutritional basis of, symbiosis among the more common microorganisms in the mouth.

3. To employ more fastidious organisms, such as spirilla, various filamentous forms and spirochetes, in order to discover less fastidious microbes whose products are required by, or enhance, the growth of the more exacting types.

Methods Employed:

1. The principal method is a modified auxanographic technique. It employs suitably formulated culture media, seeded with a selected bacterium which serves as an indicator of reaction. Standard amounts of test culture suspensions are pipetted

art A (continued)

to sterile filter paper disks which are then placed on the surface of the seeded medium. Controls are effected by the use of appropriate reference solutions in place of the test culture. Thus, quantitative as well as qualitative data are obtainable.

2. Standard or modified chemical, physical or microbiological methods are used to identify common metabolites. Specifically used for current work were:

A. Quantitative Sakaguchi reaction for arginine.

B. The pheonol-hypochlorite test of Williams for ornithine and other amines.

C. Nessler's reaction for free NH_3.

D. The hypochlorite oxidation test of Feigl for α-amino acids.

E. The adsorption on caroxite and gravimetric estimation of CO_2.

Major Findings:

1. The growth stimulating action of an oral diphtheroid on Borrelia vincentii has been described previously. Cocarboxylase and acetyl phosphate have been shown to substitute for the diphtheroid in effecting the stimulation. Cocarboxylase is about 100 times more active than acetyl phosphate. Either will support spirochetal growth in a medium devoid of both compounds. Using Bandurski's method for phosphate esters, a compound chromatographically similar to acetyl phosphate has been discovered in buffered saline (pH 7.0) extracts of ground diphtheroids. Compounds chromatographically similar to cocarboxylase were not demonstrable.

2. The partially defined medium described previously has been used for preliminary studies of the physiology of B. vincentii. Thus far, indole and NH_3 have been identified as metabolic end-products, and indirect evidence indicates that lactic acid may be produced also. With respect to NH_3 production, the guanido group of L-arginine was revealed as the primary source. Glutamine is deaminated also, but at a much lower rate. Since the arginine dihydrolase system of the strepto-

Part A (continued)

cocci is known to be exergonic, the demonstration of such a system in a spirochete might aid in explaining its energy pathways. If an arginine dihydrolase were active, then each mole of arginine should be degraded to 2 moles of NH_3, 1 of CO_2, and 1 of L-ornithine, with no intermediate formation of urea.

Experimentally, resting cell suspensions of B. vincentii degraded 9.6 of an original 15 µmoles of L-arginine per ml. Recovery of 4.9 µmoles of NH_3, 1.6 µmoles of CO_2, and 1.55 µmoles of ornithine accounted for approximately 1.6 µmoles of arginine. There was no evidence of urea formation. The remaining 8 µmoles of arginine were probably degraded to an amine, since they were accounted for by the phenol-hypochlorite test, but not by the hypochlorite oxidation test which is specific for α-amino acids according to Feigl. The ratio of CO_2 to ornithine produced is about 1, which is theoretical for an arginine dihydrolase system. The 8 µmoles of CO_2 and the 14.3 µmoles of NH_3 unaccounted for are probably used in the synthesis of other compounds.

Significance to Dental Research:

1. Studies such as that herewith reported are natural sequelae to those concerning the nutrition of single organisms. Knowledge of the nutritional requirements of multiple cultures will eventuate in the more complete understanding of biological microcosms.

2. An understanding of the mechanisms whereby the nature of the oral flora is determined in health, will enable the more certain recognition of changes therein due to, or having etiological significance in, oral diseases.

Proposed Course of Project:

1. The studies of the arginine metabolism of B. vincentii will be continued. Of particular interest is the identity of the suspected amine, and the fate of the CO_2 and NH_3 not recovered.

2. Further studies of spirochetal physiology are planned. Preliminary work on sulfur and carbohydrate metabolism suggest that these approaches may be fruitful.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Hampp, E. G., and Nevin, T. A. Substitution of known compounds for ascitic fluid in the cultivation of Borrelia vincentii. J. Bacteriol., 77:800-803, 1959.

Nevin, T. A., and Hampp, E. G. Partially defined medium for the cultivation of Borrelia vincentii. J. Bacteriol., 78:263-266, 1959.

Honors, and Awards relating to this Project:

None

Serial No. NIDR-[illegible]
2. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Bacteremias Following Exodontia and Periodontal Treatment.

Principal Investigator: Dr. T. A. Nevin

Other Investigators: Mr. M. Rogosa
Dr. E. G. Hampp
Dr. R. J. Fitzgerald
Dr. A. Howell, Jr.
Dr. H. V. Jordan

Cooperating Units: American Dental Association

Man Years (calendar year 1959):
Total: 3/4
Professional: 1/4
Other: 1/2

Objectives:

To relate those organisms isolated from the peripheral blood during dental surgery to those isolated from pathological materials obtained from the site of surgery in the same patient. This is a continuation of NIDR-26 (1957).

Methods Employed:

1. Standard bacteriological methods, including 6 tolerance tests, 11 chemical tests, and 26 fermentation reactions, are being used to identify the organisms isolated from oral samples.

2. Upon completion, these data will be compared with those obtained similarly from the study of blood isolates in order to show any relationships.

Part A (continued)

Major Findings:

1. Approximately 180 strains, isolated from blood, have been characterized.

2. Approximately 250 strains, isolated from oral samples, are being processed. Among these are about 100 strains of streptococci and diphtheroids isolated in Dr. Fitzgerald's laboratory, and 30 strains of micrococci isolated in Dr. Jordan's laboratory. An additional 30 strains of filamentous organisms have been isolated and characterized in Dr. Howell's laboratory.

Significance to Dental Research:

In the event that a sufficiently high proportion of oral isolates prove identical with blood isolates obtained from the same patient, these data should be valuable in designing future studies of the immunological and physiological aspects of infection in dental, and related diseases.

Proposed Course of Project:

1. It is anticipated that the laboratory work will be completed by December 15, 1959.

2. The data will then be analyzed to compare the oral and blood isolates.

3. Upon completion of the analysis, the data will be put into final publication form.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Rogosa, M., Hampp, E. G., Nevin, T. A., Wagner, H. J., Driscoll, E. J., and Baer, P. N. Blood Sampling and Cultural Studies in the Detection of Postoperative Bacteremias. J. Am. Dent. Assoc., in press.

Honors and Awards Relating to this Project:

None.

Serial No. [illegible]
1. Microbiology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on the Physiology of Oral Fusobacteria and on Proteolytic Enzymes of Microbial Origin

Principal Investigator: Dr. R. R. Omata

Other Investigators: Dr. E. G. Hampp

Cooperating Units: American Dental Association

Man Years (calendar year 1959):
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives:

To investigate the physiology of various members of the oral microbial flora and the nature of their products, specifically proteolytic enzymes, as factors in periodontal disease.

Methods Employed:

1. Standard procedures for the isolation, classification, and investigation of the nutritional requirements of bacteria were employed. Biochemical activities were correlated with cellular and colonial morphology to differentiate types commonly found in the oral cavity.

2. Extracellular bacterial proteases were fractionated by salting out with ammonium sulfate and by chromatographic methods using carboxymethylcellulose columns. Proteolytic activity on various types of substrates was measured by the decrease in total protein and the increase of α-amino acids. In growing cultures, proteolysis was correlated with direct cell counts at various stages of growth.

Major Findings:

1. Studies on the isolation, classification and nutrition of oral fusobacteria were continued. Among the 25 strains of oral fusobacteria investigated, three types or species were found. Fusobacterium nucleatum and F. polymorphum were very similar in their heterofermentative processes, biochemical reactions and colonial morphology but different in cellular morphology. The third type, F. plauti-vincenti, a homofermentative type, is very different from the others in morphology and physiology.

2. The nutritional requirements for optimal growth of the Fusobacterium nucleatum types were found to be casein hydrolyzate, tryptophan, glucose, pantothenate and/or pantothenol, pantetheine and Coenzyme A, in addition to adenine, hypoxanthine, and 4-amino, 5-imidazole carboxamide. Casein hydrolyzate could not be replaced by various combinations of 19 pure L-amino acids.

3. By chromatography on carboxymethylcellulose columns with pH gradient elution from pH 4.6 to 8.5, it has been possible to obtain three fractions, active on bovine collagen, from ammonium sulfate precipitated extracellular proteases produced by Clostridium histolyticum. It has been possible to purify the "collagenase" activity by 5 to 10-fold with this method; however, there are indications of heterogeneity of proteolytic activity of certain fractions.

4. The proteolytic activity of certain oral spirochetes was investigated during growth in Huntoon's broth with and without ascitic fluid. Present data show that the small oral treponeme proteolyzes actively during its early growth phase; in addition, the liberation of free amino acids indicates strong peptidase activity. Borrelia vincentii showed definite though lesser proteolytic activity during its growth phase.

Significance to Dental Research:

The present investigation is based on the concept of the role of different groups of microorganisms and their metabolic products as contributory factors in the initiation or aggravation of primary periodontal disease. There are implications that various bacteria and their enzymes may have an integral relationship in the etiology of oral diseases, and a basic understanding of these factors should be a fruitful approach.

Part A (continued)

Proposed Course of Project:

1. Further work will be conducted on the nutritional requirements of the oral fusobacteria, in particular the role of purines and purine analogues, which may enhance or inhibit growth of the fusobacteria and affect the metabolism of adenine and hypoxanthine.

2. The fractionation studies of "collagenase" will be extended to differentiate the three active fractions and to investigate the site and specificity of activity on collagens from several different sources.

3. The studies on the proteolytic activity of the oral spirochetes will be extended to investigate the various types of protein substrates they are capable of utilizing for growth and to study adaptability of certain strains to assimilate simpler forms of derived proteins.

Part B included		Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Omata, R. R. Studies on the Nutritional Requirements of Fusobacteria. II. Requirements for Pantothenate and Purines. J. Bacteriol., 77: 35-37, 1959.

2. Hampp, E. G., Mergenhagen, S. E., and Omata, R. R. Studies on Mucopolysaccharase Activity of Oral Spirochetes. J. Dental Res., 38: 979-982, 1959.

Honors and Awards Relating to this Project:

None

Serial No. [illegible]
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Methods of Using the Hamster Cheek Pouch
for Periodontal Research

Principal Investigator: Dr. A. A. Rizzo

Other Investigators: Dr. S. E. Mergenhagen
Dr. E. G. Hampp

Cooperating Units: American Dental Association

Man Years (calendar year 1959):
Total: 1/4
Professional: 1/4
Other: 0

Project Description:

Objectives:

Since the hamster cheek pouch allows microscopic visualization of living oral tissue, it provides a useful system for studying the effects of oral microorganisms and their products. Accordingly, the general objectives of this project were to modify present cheek pouch techniques so that they could be used readily in the average microbiology laboratory, and to begin applying these methods to specific problems.

Methods Employed:

One or both cheek pouches of anesthetized hamsters were stretched and fastened without rupturing epithelium onto a wooden board constructed to allow observation by both transmitted and reflected light. Preparations for high magnification required removal of a portion of one wall of the pouch. In such specimens, either the submucous blood vessels or intact epithelium could be faced upward for access. Zweifach's methods of maintaining the exposed tissue and testing its suitability (epinephrine threshold) were used. Endotoxins from a strain of *Veillonella* and

Part A (continued)

from Selenomonas sputigena in various dilutions in Ringer's solution at pH 7.4 and 37°C. were tested for direct effects on capillaries. Under magnification of 10X, Veillonella and spirochete cultures, bacteriologic medium, saline, and turpentine were injected with a 30 gauge needle into intact pouch walls. Reproducible blebs of 3 mm. were obtained with 0.01 ml. of these substances, and the immediate and delayed tissue reactions observed. In order to allow detailed study of pouch tissue subjected to various procedures in vivo, methods of preparing stained mounts were tested and a suitable procedure developed.

Major Findings:

Endotoxins (1:200) from oral organisms have not been demonstrated thus far to have immediate effects on exposed capillaries of the hamster pounch. A small inoculum of oral spirochetes in the pouch wall caused lesions basically similar to those produced recently in rabbit skin by Hampp and Mergenhagen. Whole mounts of experimental pouch tissue were best prepared when fixed immediately by immersion in concentrated formalin, defatted in dioxane, stained in Lillie's azure-eosin, and dehydrated under tension in acetone.

Significance to Dental Research:

The significance of this preliminary work lies in the demonstration that the hamster cheek pouch can be used profitably with routine microscopic equipment and a small amount of trial effort.

If confirmed, the preliminary finding that oral endotoxins do not directly affect microcirculation may be an important consideration in interpretations of periodontal disease mechanisms.

That spirochetal infections can be produced in this tissue complements recent work on the pathogenicity of these organisms.

Part A (continued)

Proposed Course of Project:

Different preparations of endotoxins from oral organisms will be continually tested for immediate effects and also for delayed effects upon capillary circulation. Attempts to induce the Shwartzman reaction in the cheek pouch will be made. A system for testing the invasiveness of oral organisms into epithelium has been designed and will be tested.

Part B included No

Serial No. [illegible]
1. Pathology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: A Comparison of the Pathology of Chronic Magnesium Deficiency and of Chronic Dilantin Sodium Toxicity in Rats

Principal Investigator: Dr A. A. Rizzo

Other Investigators: Dr. H. R. Stanley, Jr.

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/2
Professional: 1/2
Other:

Project Description:

Objectives:

1. To compare and contrast the pathology of chronic magnesium deficiency and chronic dilantin toxicity in rats by histological and biochemical means.

2. To determine whether the gingival hyperplasia described in magnesium-deficient rats resembles human dilantin hyperplasia clinically and histologically. Hitherto it has not been possible to produce dilantin gingival hyperplasia in any animals except ferrets.

3. To determine whether gingival overgrowth can be produced in rats on a diet containing marginal amounts of magnesium and toxic amounts of dilantin.

4. To determine whether chronic dilantin intake alters mineral metabolism in certain tissues.

[illegible] (continued)

Method Employed:

1. Groups of 15 female Sprage-Dawley rats 2-3 months old were placed for periods ranging from 26 to 150 days on 5 dietary regimens. The respective diets contained:

I. Optimal concentration of magenesium (0.05%);
II. Marginal concentration of magnesium (0.01%);
III. Deficient concentration of magnesium (0.00+%);
IV. Toxic concentration of dilantin sodium (1.0%) with optimal concentration of magnesium; and
V. Toxic concentration of dilantin with marginal concentration of magnesium

Both magnesium-deficient and dilantin toxic animals were finally placed on curative (normal) diets for periods as long as 50 days.

2. *In vivo* oral examinations were performed with the aid of a newly developed mouth prop for rodents. *Post mortem*, tissues were fixed for histologic examination and frozen for biochemical study. Hematocrit analyses and blood counts were performed by routine methods. Calcium, magnesium, and phosphorus contents of mandibles and femurs were determined by methods used by McCann. Skin collagen was measured by Martin and Axelrod's method. Measurements of fixed gingivae were performed with an instrument designed by Keyes.

Major Findings:

1. Rats on diets I and II showed no obvious abnormalities. Rats on diet III developed after 2 to 3 months classical deficiency signs, including a marked gingival hyperplasia which resembled human dilantin hyperplasia. Rats on diet IV showed a slight increase of gingival bulk; and a few rats on diet V for 5 months developed slight gingival hypertrophy. Under a curative dietary regime the fibrous overgrowth of magnesium-deficient rats regressed completely within 7 weeks.

2. Although a decrease in tibial ash values was noted in rats on diets II and IV, a small number of tibial and mandibular bone analyses showed similar calcium values in all groups, and decreased magnesium values only in the magnesium-deficient group (diet III).

Part A (continued)

3. A hitherto unreported anemia, not related to weight loss, was found in rats magnesium-deficient for 3 months. Preliminary histologic survey implicates both medullary depression and excess hemolysis in the mechanism of this anemia.

Significance to Dental Research:

This project provides information related to a gingival condition which causes premature loss of teeth in many patients, and gives data on the general pathologic effects of a widely used, chronically ingested drug.

The extreme fibrous overgrowth of free gingivae which occurs in magnesium deficiency, but not in any other experimental condition in rats, must surely have fundamental significance regarding gingival metabolism and fibroplasia.

The finding that magnesium-deficient rats develop anemia may be related to the reported observation that normal red blood cells *in vitro* suffer increased fragility when magnesium is restricted.

Proposed Course of Project:

The histopathologic and biochemical studies on the material from this project are to be completed as described. Evaluation of the total data thus obtained will give direction to future efforts.

Part B included   Yes

Serial No. NIDR-31

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Rizzo, A. A. A New Mouth Prop for Oral Examinations and Operative Procedures in Rodents. J. Dental Res., 38: 830-832, 1959.

Honors and Awards Relating to this Project:

None

Serial No. NIDR-32
1. Microbiology
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Immunological, Biochemical, and Pathological Studies of Oral Bacteria

Principal Investigator: Mr. M. Rogosa

Other Investigators: None

Cooperating Units: National Institute for Research in Dairying, University of Reading, Shinfield, Reading, England

Man Years (calendar year 1959):

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

To determine the immunological, biochemical, and pathological characteristics of the veillonellae, lactobacilli, diphtheroids, and other oral microorganisms. This project is a continuation of NIDR-22 (1958).

Methods Employed:

1. Antisera, prepared in rabbits against bacterial cells, are tested against the organisms or antigens extracted from them by the techniques of agglutination, precipitation, and selective antibody absorption.

2. Biochemical characteristics are determined by standard and newly developed procedures.

3. Pathogenicity studies are conducted in appropriate animal hosts, inoculated by various routes.

Part A (continued)

Major Findings:

1. Serology: During the calendar year 1959, studies on the veillonellae, previously described in the report for the calendar year 1958, were suspended. These studies will be recommenced after the principal investigator's return to the National Institutes of Health in 1960. Also, the experimental data, which are already sufficiently complete, will be presented for publication at that time. Serological studies on the lactobacilli, particularly strains isolated from the hamster, are now complete and appropriate revised manuscripts, incorporating comprehensive findings, will be submitted for publication. In addition, Lactobacillus salivarius, a new species described by the principal investigator, has been found to possess distinctive serological properties.

2. Pathogenicity: No animal pathogenicity studies were done this year. However, studies on postoperative bacteremias in human patients, in which 82% of extraction cases and 88% of periodontal patients had postoperative bacteremias and in which improved technical control contibuted to this high recovery of diverse and often multiple organisms in individual bacteremias, have been described in a manuscript accepted for publication by the Journal of the American Dental Association.

3. The chief purpose of the principal investigator's work in England, in cooperation with Dr. M. Elisabeth Sharpe, of the National Institute for Research in Dairying, University of Reading, Shinfield, Reading, has been to attempt to clear up existing misunderstandings concerning the lactobacilli, to determine optimal conditions for appropriate tests, and to arrive at a natural system for the determination of species identity within the genus Lactobacillus acceptable to European and American workers. In the leading lecture in a symposium on the lactobacilli held by the Society for Applied Bacteriology in London, on July 7th, 1958, we described how this has been largely achieved. A manuscript presenting our conclusions has been accepted for publication in the Journal of Applied Bacteriology.

A study of vaginal lactobacilli, "Döderlein's bacillus," has demonstrated that, in reality, various species are present.

Part A (continued)

Previously studied representative diphtheroids from the rat have been examined by means of the chromatographic technique developed by the English group for the analysis of cell components, particularly amino acids and peptides. Both catalase-negative and catalase-positive varieties have been employed, but present results indicate only quantitative rather than qualitative differences in the total amino acid pool.

Significance to Dental Research:

Since systematic immunological and pathogenicity studies are lacking, these studies are important in differentiating organisms; in increasing knowledge of the structure of the cell; and in determining the pathogenic potential of the mouth microbiota.

Proposed Course of Project:

The immediate future will be devoted to the immunology and chemical composition of the lactobacilli and their taxonomic relationship to the oral diphtheroids, particularly the catalase-negative diphtheroids; and to the serological characteristics of streptococci isolated previously from the rat. Further antigenic and pathogenic analysis of the *Veillonella* is required, particularly of the animal strains. Present techniques will be adapted for precipitin studies and the use of conjugated labeled antibodies for the demonstration of the organisms *in situ* in tissues.

Part B included Yes

PHS-NIH
Individual Project Reports
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Rogosa, M. and Sharpe, M. E. An Approach to the Classification of the Lactobacilli. J. Appl. Bact., in press.

Rogosa, M., Hampp, E. G., Nevin, T. A., Wagner, H. M., Driscoll, E. J., and Baer, P. N. Blood Sampling and Cultural Studies in the Detection of Postoperative Bacteremias. J. Am. Dent. Assoc., in press.

Honors and Awards Relating to this Project:

Resident Visiting Scholar, University of Reading

Guest Lecturer, University of London

Serial No. [illegible]
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Manifest and Inapparent Viral Infections of Oral Tissues

Principal Investigator: Dr. H. W. Scherp

Other Investigators: Dr. R. J. Fitzgerald

Cooperating Units: None

Man Years (calendar year 1959):
Total: 3
Professional: 1/2
Other: 2 1/2

Project Description:

Objectives:

1. To test for the presence of inapparent viruses in the tissues of "germfree" and conventional animals.

2. To isolate, identify, and determine the etiological significance of viruses from various lesions of the oral mucosa of undetermined etiology.

This project is a continuation of NIDR-12 (1958).

Methods Employed:

Samples containing tissue cells are frozen and thawed to liberate intracellular viruses. To test for viruses, the samples are inoculated into standard tissue cultures of rabbit kidney cells, monkey kidney cells, Hela cells, L cells, chicken embryo cells, and human skin cells. After incubation, cultures are frozen, thawed, and transferred to a fresh tissue culture; serial transfers are made. In addition, specimens of biopsy tissue are maintained in tissue culture medium for protracted periods

Part A (continued)

to allow development of latent viruses. Evidence for a virus is sought by microscopic examination for cytopathologic changes in the cultured cells and by hemagglutination and hemadsorption tests of the culture fluids with guinea pig and chicken erythrocytes. Isolates of viruses are identified by neutralization tests with specific antisera, when applicable.

Major Findings:

1. Cytopathogenic agent from rat salivary glands. It was reported previously that extracts of salivary glands of a majority of both germfree and conventional rats contained an agent cytopathogenic for certain animal cells in culture. The character of the cytopathologic effect (CPE) and evidence of transmissibility in cell cultures indicated that the agent might be a virus. The recent program has aimed to substantiate this possibility.

a. Incidence of agent. The agent was demonstrable, in dilutions as high as 10^{4} - 10^{5} (tissue = unity), in the salivary glands of 22 of 31 germfree rats and 6 of 7 conventional rats. Nearly 200 samples of throat and rectal swabs, urine, brain, kidney, pancreas, liver, lung, and spleen from germfree rats were not cytopathogenic. Extracts of the feces of these animals were highly toxic for cell cultures but the effect was quite different from the CPE of the salivary gland agent and not transmissible from culture to culture.

b. Range of susceptible cells. Monkey kidney cells, Hela cells, and chicken embryo fibroblasts were insusceptible. Rabbit kidney cells were affected very rapidly. The CPE began within 16 hours at widely separated foci in the cellular sheet as a rounding of several cells in juxtaposition, which subsequently coalesced to an enlarged rounded form that joined similar nearby cells in grape-like clusters. This reaction spread throughout the sheet with essentially complete detachment of the affected cells from the vessel wall within 24 to 36 hours. Human skin cells behaved similarly except that about 72 hours were required for

Part A (continued)

full development of the CPE. Recent results with mouse fibroblasts, strain NCTC#L-929, are most promising. Their cytopathologic reaction resembles that produced by herpes virus, with the formation of very pleomorphic giant forms that are stable and have continued to multiply without reversion to the original form during 6 transfers at biweekly intervals.

c. Serial transmission. In several series of experiments, cultures of rabbit kidney and human skin cells were allowed to develop the CPE fully and aliquots of the cell-free culture fluids were transferred to fresh homologous cell cultures. The CPE was manifest through 4 to 5 such transfers in series, equivalent to a final dilution of inoculum that should rule out carry-over of a nonreplicating toxic factor. Furthermore, this CPE was more rapid and extensive than that produced by higher dilutions of the original salivary gland extract. Similarly, when L-929 cells were exposed to the agent and subcultured serially, the CPE was produced on rabbit kidney cells by the supernatants after 3 subcultures.

d. Adsorption of agent by susceptible cells. In a number of experiments, susceptible cells have been exposed to various dilutions of different salivary gland extracts for 1/2 and 1 hour at 4° C. and 37° C., washed with culture medium, and incubated. Such cells developed the CPE typically. These results are consistent with the action of a virus, though not conclusive proof of one.

e. Neutralization tests. The CPE of 4 rat salivary gland extracts was reduced or prevented by inclusion in the medium of pooled serum from germfree rats, affected slightly by normal calf serum, and prevented by normal rabbit serum in 2 instances.

f. Miscellaneous. Hemagglutination and hemadsorption tests for virus in salivary gland extracts and in supernatants of cell cultures undergoing the CPE have been regularly negative. Microscopic examination of cells exhibiting the CPE and stained by Giemsa's method has revealed no inclusions. It has not been possible to produce viral plaques in cell cultures with the salivary gland agent. Injection of rat salivary gland extracts intracerebrally and intraperitoneally into suckling and adult mice and young and old rats has produced no pathologic effect.

Part A (continued)

2. Serologic differentiation of strains of herpes simplex virus. With rare exceptions, strains of herpes simplex virus have been found to be antigenically identical. In view of the ubiquity of this virus in the oral area, a serological differentiation of strains should have both theoretical and practical significance. Such a differentiation is being accomplished by measurement of the rate constants of neutralization by homologous and heterologous antisera, a method previously applied with success to polioviruses by Dr. W. D. McBride in this Laboratory. Accurate enumeration of virus by plaque formation in cell culture is essential. It has been necessary to work out optimal media and cultural conditions for plaque formation by herpes simplex virus in cultures of rabbit kidney cells. Four stock strains and 11 recently isolated strains of herpes simplex virus and homologous rabbit antisera are available from a previous investigation in this Laboratory (NIDR-12 (1958)). To date, the neutralization of 8 strains by 4 antisera has been studied. The results show that a clear-cut grouping of strains is feasible.

Significance to Dental Research:

Oral soft tissue lesions of unestablished but possibly viral etiology are frequently encountered by the dentist. The still incompletely understood phenomena of latency and activation of herpes virus are accordingly of direct dental interest; they also provide prototypes of fundamental viral relationships. Study of inapparent viral infection in otherwise germfree animals affords another approach to the study of the consequences of such infections on "normal" physiology.

Proposed Course of Project:

The previously described studies of cytopathogenic agent from rat salivary glands and of serological differentiation of strains of herpes virus will be continued.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

McBride, W. D. Antigenic Analysis of Polioviruses by Kinetic Studies of Serum Neutralization. Virology, 7: 45-58, 1959

Honors and awards relating to this project:

None

[illegible]
1. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Digestion of Native and Altered Collagens by Oral Microorganisms in Relation to Periodontal Disease.

Principal Investigators: Dr. H. W. Scherp
Dr. S. E. Mergenhagen

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 2 1/2
Professional: 3/4
Other: 1 3/4

Project Description:

Objectives:

1. To determine whether members of the oral microbiota, singly or in combination, can digest native collagen.

2. To determine whether collagen altered in vivo, as by inflammation, becomes more susceptible to digestion by the oral microbiota.

3. To determine whether collagen altered in vitro, as by temperature or pH of environment, or both, becomes more susceptible to digestion by the oral microbiota.

This project is a continuation of NIDR-11 (1958).

Methods Employed:

Animal skin was selected as a collagenous substrate resembling gingival tissue in essential features. Areas of inflammation are induced by four methods: a sterile (turpentine) abscess; infection, as by staphylococci and mixed infection with oral microorganisms; cutaneous application of irritants such as

Part A (continued)

xylene and croton oil; and allergic reaction of sensitized animals to cutaneous application of a simple allergen, such as picryl chloride. Areas of normal and inflamed skin from each animal are defatted, dehydrated, and sterilized by immersion in acetone. An an ancillary substrate, collagen fibrils are reconstituted from the acid-soluble collagen of such specimens. Samples are exposed to cultures in a special medium, inoculated with accumulations from human periodontal pockets or organisms isolated from such cultures. Residual tissue and microbial growth are removed by centrifugation. Collagenolysis is estimated by comparing the hydroxyproline contents of the undigested residues with those of control samples not exposed to the cultures.

Major Findings:

1. Revision of methods. Enzymatic digestion of collagen is most conveniently measured by the liberation of soluble products containing hydroxyproline, which is a specific constituent of collagen. Applicability of this procedure to digestion by growing microbial cultures, however, was found to be limited by the fact that a number of microorganisms destroyed these specific products, often as rapidly as they were formed. Accordingly, the procedure was modified to determine decrease of collagen rather than liberation of hydrolytic products. For present investigations, this approach requires a homogeneous, unaltered collagenous substrate that can be dispensed quantitatively and aseptically; unfortunately it is therefore not readily applicable to intact tissue. On the other hand, it has been used very successfully with collagen fibrils reconstituted from the acid-soluble collagen of rabbit skin.

2. Bacteriolysis of reconstituted collagen. Nineteen of 26 broth cultures of gingival scrapings from 10 subjects lysed reconstituted collagen from rabbit skin at varying rates. This is believed to be the first definitive demonstration of lysis of an undenatured collagen by the oral flora. However, this substrate resisted the individual and combined action of the following cultures isolated from such mixtures: 7 strains of gram-negative anaerobic cocci (genus *Veillonella*), 6 strains of *Fusobacterium*, 4 strains

Part A (continued)

of streptococci, and 2 strains of diphtheroid bacilli. These results support the often advanced hypothesis that more than one species of bacteria are involved in the degradation of collagen in periodontal disease. They supplement previous indications of a symbiotic effect of mixed oral cultures in lysing "collagen paper" or altered collagen in tissue sterilized by ethylene oxide. Additional evidence of such relationships has been provided by the demonstration that whereas only 6 of the pure cultures listed above digested only to a slight extent reconstituted collagen that had been treated with ethylene oxide, the combination of all 15 cultures digested it readily. Concomitantly with increased digestibility from treatment with ethylene oxide, reconstituted collagen exhibited marked morphological changes, as revealed by electron microscopy, analogous to those reported to occur in areas of inflammation. These observations encourage further exploration of the possibility that collagen can be rendered more digestible by physiological processes.

3. Bacteriolysis of collagen in tissue. Although entirely satisfactory methods of substrate preparation have not been developed as yet for measuring the digestion by growing bacterial cultures of collagen in tissue, significant results have been obtained using dried defatted skin, comminuted in a mill and sterilized by acetone. Such preparations from rabbit skins inflamed by chemical irritants (xylene and turpentine) were digested by a proteolytic *Bacillus* species, whereas a preparation of normal skin from the same animal underwent no collagenolysis. However, owing to imperfections in the present method, it has not yet been possible to ascertain whether or not the oral flora can attack collagen under similar conditions.

Significance to Dental Research:

Degenerative changes of collagen fibers in the marginal gingivae are a prominent feature of periodontitis. Disruption of the collagenous fiber attachment of the root of the tooth to the alveolar bone with eventual loss of the tooth is the most important consequence of periodontal disease. It is essential, therefore, to determine whether such collagenolysis results from the inflammatory reaction *per se*, from proteolysis by the oral microbiota, or from a combination of these processes.

Part A (continued)

Proposed Course of Project:

Attention will be directed (a) to determine which members of the oral flora effect the digestion of altered and unaltered reconstituted collagen *in vitro*; (b) to improve methods to assay lysis of collagen in tissue by growing cultures of the oral flora; and (c) to apply such improved methods to compare the digestibility of collagen in normal and inflamed tissue.

Part B included	Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Mergenhagen, S. E., and Scherp, H. W. Lysis of Reconstituted Collagen and Catabolism of Products of Collagenolysis by the Oral Microbiota. Arch. Oral Biol., in press.

Mergenhagen, S. E., Scott, D. B., and Scherp, H.W. Lysis by Human Oral Bacteria of Collagen Altered by Ethylene Oxide. Proc. Soc. Exptl. Biol. Med., in press.

Honors and Awards Relating to this Project:

Invited participant (H.W. Scherp), Josiah Macy, Jr. Foundation Conference on Polysaccharides in Biology, June 1959.

Invited speaker (H.W. Scherp), International Conference on Oral Biology, (International Association for Dental Research) September 1959.

[illegible]
2. Microbiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Nutrition and Metabolism of Lactic Acid Bacteria.

Principal Investigator: T. Shiota

Other Investigators: None

Cooperating Units: American Dental Association

Man Years (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To study amino acid and vitamin metabolism by lactic acid bacteria; with specific attention to enzymatic synthesis of folic acid-like compounds by purified extracts of *Lactobacillus arabinosus*.

Methods Employed:

The determination of folic acid-like compounds was carried out by microbiological assay, chromatography, and spectrophotometry. The purification of enzymes in bacterial extracts was performed by ammonium sulfate fractionation and cellulose chromatography.

Major Findings:

1. It was previously reported that sonic extracts of *L. arabinosus* synthesized a folic acid-like compound from p-aminobenzoylglutamic acid and reduced 2-amino-4-hydroxy-6-hydroxymethylpteridine (pteridine). This reaction required magnesium and adenosinetriphosphate

Part A (continued)

(ATP). We have since obtained information on the nature of the folic acid-like compound and the reduced pteridine. The folic acid-like compound behaved like tetrahydrofolic acid when assayed bioautographically by Pediococcus cerevisiae in the presence of a reducing agent. Like tetrahydrofolic acid, it was rapidly oxidized in the absence of a reducing agent. When it was formylated, either chemically with formic acid or enzymatically with transformylase, 5-formyltetrahydrofolic acid (citrovorum factor, a stable derivative) was obtained, as would be expected from tetrahydrofolic acid. This result was ascertained microbiologically and chromatographically.

2. Other evidence indicated that the reduced pteridine was 2-amino-4-hydroxy-6-hydroxymethyl tetrahydropteridine: (a) a mole of pteridine consumed four atoms of hydrogen by catalytic hydrogenation, and (b), one mole of reduced pteridine contained four equivalents of hydrogen as determined by iodometric titration. The ultraviolet spectral characteristic of the tetrahydropteridine shows that at pH 3, the absorption maximum occurs at 265 mμ. Values for molar extinction coefficient varied between 11,000 and 13,000. The tetrahydrofolic-acid-synthesizing enzymes in the bacterial extract have been purified 25-fold by ammonium sulfate fractionation and diethylaminoethyl-cellulose chromatography. Preliminary experiments indicate that the role of ATP is to phosphorylate the tetrahydropteridine since a chemically phosphorylated reduced pteridine was active in replacing ATP, plus tetrahydropteridine, in tetrahydrofolic acid synthesis.

Significance to Dental Research:

Lactic acid bacteria have long been associated with dental caries and probably their importance may lie in their ability to grow rapidly and produce acid. The basic study of nutrition and metabolism of lactic acid bacteria should contribute to a better understanding of the microbial activities in the oral cavity.

Proposed Course of Project:

The work on folic acid synthesis is to be continued. It is anticipated at this time that efforts will be directed toward obtaining information concerning the role of ATP.

Part B included Yes

Serial No. [illegible]

PHS-NIH
Individual Project Reports
Calendar Year 1959

Part B: Honors, Awards, and Publications.

Publications other than abstracts from this project:

Shiota, T. Enzymic Synthesis of Folic Acid-like Compounds by Cell-free Extracts of *Lactobacillus arabinosus*. Arch. Biochem. Biophys., 80: 155-161, 1959.

Disraely, M. N., Shiota, T., and Caplow, M. The Occurrence and Origin of Certain Vitamins in Saliva. Arch. of Oral Biology, in press.

Honors and Awards Relating to this Project:

None

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1959

BIOCHEMISTRY:

26. F. J. McClure: Nutrition and Dental Caries with Emphasis on Cereal Foods, Lysine, Calcium and Phosphate Supplements.

27. I. Zipkin: Biochemistry and Physiology of the Salivary Glands and their Secretions.

28. I. Zipkin: Non-dental Physiological Effects of Fluoride.

29. H. G. McCann: The Inorganic Chemistry of Dental Tissues, Surface Enamel Chemistry as Affected by Diet and Oral Environment, and their Relation to Dental Caries.

30. J. E. Folk: Protein and Amino Acid Chemistry. I. Proteolytic Enzymes; Chemical and Molecular Studies, Isolation, Purification and Kinetics. II. Blood Clotting; Enzymology, Metabolism and Molecular Mechanism.

31. M. S. Lewis: Physical Chemistry of Proteins.

32. M. S. Lewis: Biochemistry of Salivary Proteins.

33. R. C. Likins: Biochemical and Biophysical Studies of Calcification.

34. K. A. Piez: Analytical and Structural Aspects of Collagen in Relation to Function and Disease.

35. K. A. Piez: Biochemistry of Amino Acids and Proteins in Mammalian Cell Cultures.

36. R. H. Larson: The Role of Tetracycline in Experimental Caries in the White Rat.

37. H. Blumenthal: Metabolism of Oral Flora and Salivary Glands. (A) Nucleoside Metabolism in Rat Submaxillary Glands; (B) Hexosamine Metabolism of Microorganisms.

38. G. R. Martin: Studies on Collagen. I. Biochemical Studies on Collagenolysis. II. Relation of Amino Acid Composition and Reactivity to Polymer Form.

Serial No. [illegible]
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Nutrition and Dental Caries with Emphasis on Cereal Foods, Lysine, Calcium and Phosphate Supplements.

Principal Investigator: Dr. F. J. McClure

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	4 1/4
Professional:	1
Other:	3 1/4

Project Description:

Objectives:

To obtain information on (a) the relation of heat processed and cereal foods, to the development of experimental rat caries, and (b) the effect of calcium and phosphate-minerals and protein supplements, on experimental dental caries.

Methods Employed:

1. The quantity and quality of protein in the diet are varied to determine their relation to the development of experimental rat caries.
2. Special diets containing common cereals of varying protein quality, as well as different mineral phosphates, calcium salts, and protein supplements, are evaluated in the development of experimental caries in white rats.

Major Findings:

1. Additional data were obtained this year to verify the cariostatic effect of phosphate supplements. Not all

phosphates have proved to be cariostatic and the data suggest differences in ortho, meta and pyro phosphates That the relative availability of a phosphate has a major influence on its cariostatic effect had continued support.

2. The solubility of a calcium mineral supplement, as a factor in reducing caries, also has been substantiated by this year's research.

3. The importance of lysine as an anticaries factor when incorporated in a lysine deficient millet-base diet was demonstrated.

Significance to Dental Research:

The continuing evidence which indicates that a soluble phosphate mineral, and possibly a soluble calcium mineral are cariostatic, suggests that some fundamental inhibitory reaction occurs possibly on enamel surfaces, within the oral cavity. This effect has interesting implications relative to the control of dental caries by oral therapy. The role of cereal foods as cariogenic agents in human diets is of continuing interest in the light of our continuing results with different cereal foods and lysine deficient diets.

Proposed Course of Action:

Observations will evaluate experimental rat caries as influenced by various phosphate and calcium mineral supplements. A number of different cereals and the role of amino acid supplements will be studied as factors in experimental dental caries.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

McClure, F. J. and Muller, A., Jr. Further Observations on the Cariostatic Effect of Phosphates. J. Dent. Res. 38:776-781, July-Aug., 1959.

McClure, F. J. and Muller, A., Jr. The Caries Inhibiting Effect of Dibasic Sodium Phosphate and Dibasic Calcium Phosphate Added to Wheat Flour and Bread Diets. J. Am. Dent. Assoc. 58:36-41, Jan. 1959.

McClure, F. J. The Inhibition of Dental Caries by Phosphate Minerals. J. of the Indiana State Dent. Assoc. 38:394-397, Nov. 1959.

Serial No. NIDR-11
1. Biochemistry
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Biochemistry and Physiology of the Salivary Glands and their Secretions

Principal Investigator: Dr. I. Zipkin

Other Investigators: Dr. E. F. Geever

Cooperating Units: Dr. P. Pronove, Dr. P. Chen (NHI, General Med. & Exptl. Therapeutics Branch)

Man Years (calendar year 1959):

Total:	2 1/2
Professional:	1 1/4
Other:	1 1/4

Project Description:

Objectives:

To study the basic chemistry and physiology of parotid, submaxillary, and whole salivary secretions and their relation to oral and systemic disease.

Subproject A.

The tyrosine and tryptophan content of human parotid saliva as determined by differential spectrophotometry.

Methods Employed:

Chemically, tyrosine is determined according to Udenfriend and Cooper, and tryptophan according to Steers and Sevag on both the whole parotid saliva and on the TCA precipitate. The ultraviolet absorption spectra, the total protein by the Lowry procedure, and Kjeldahl nitrogen are determined on the TCA precipitate.

Major Findings:

A highly significant correlation exists between the tyrosine

and tryptophan concentration of the TCA precipitate determined chemically and spectrophotometrically, thus providing a simple method for the determination of these two amino acids in parotid salivary protein. Attempts to make similar correlations for whole parotid saliva are unsuccessful due to uric acid interference. The Warburg-Christian equation, the Lowry procedure and total nitrogen values are being applied to calculate the concentration of the TCA precipitable protein in parotid saliva.

Proposed Course of Project:

The relation of salivary tyrosine and tryptophan to caries and periodontal disease as well as to various systemic diseases may be studied.

Subproject B.

The "salivary clearance" of phosphate and inulin.

Methods Employed:

Patients undergoing urinary clearance tests for phosphate and inulin were studied. Whenever urine was obtained from the catheterized patient, parotid saliva was collected by means of the Lashley cup. Four normal individuals, 4 patients with suspected parathyroid involvement and 3 chronic "stone-formers" comprised the study.

Major Findings:

No inulin was "cleared" from the blood by the parotid gland. This was rather surprising in view of its relatively low molecular weight and its great ease of clearance by the kidney. After phosphate infusion in the controls, the urinary phosphate increased about 6-fold, the blood phosphate increased about 2-fold, and the saliva showed only about a 20 percent increase. Some individuals in the experimental group showed greater increases in the urinary phosphate clearance and in the blood phosphate, than control individuals. The salivary "clearance" of phosphate was similar in all individuals.

Significance to Dental Research:

Since saliva is in intimate contact with the teeth and other oral structures, it is important to study its chemistry in both normal and pathological states. The physiology of the secretion of parotid saliva will be

approached by simultaneously studying the concentration of various components in blood, urine and saliva under various conditions.

Proposed Course of Project:

Studies will be continued to include additional normal and pathological cases for the determination of salivary "clearance" of these and other constituents.

Subproject C:

The salivary glands in lysine-deficient young rats.

Methods Employed:

Young rats were placed on lysine deficient diets for 60 days after which the salivary glands were subjected to routine histologic examination. Whole saliva was collected from the rats and examined for total nitrogen, protein, tyrosine and apparent tryptophan (Ehrlich positive materials) content. The teeth were also scored for caries.

Major Findings:

Salivary gland growth and development was impaired at both a gross and cellular level by lysine deficiency. No other histologic changes in the tissue were noted. Total nitrogen, protein, tyrosine and apparent tryptophan in the saliva of lysine deficient rats were normal. Caries was produced on the lysine deficient diet.

Proposed Course of Project:

None

Significance to Dental Research:

Information concerning the salivary glands and their secretions in relation to the development of dental caries serves to elucidate the etiology of this disease.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Ceever, E. F., McClure, F. J., and Zipkin, I. The Salivary Glands in Lysine-deficient Young Rats. J. Dent. Res. In press.

Serial No. NIDR-19
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Non-dental Physiological Effects of Fluoride.

Principal Investigator: Dr. I. Zipkin

Other Investigators: Dr. F. J. McClure

Cooperating Units: None

Man Years (calendar Year 1959):

Total:	2
Professional:	1/4
Other:	1 3/4

Project Description:

Objectives:

To study the deposition and retention of fluoride in the calcified tissues of young and adult animals.

Methods Employed:

In two separate studies, 10 ppm F as NaF was given to 30-day old rats for either 10 weeks or 52 weeks and then withdrawn. The following calcified tissues were analyzed separately for fluoride at successive ten-week intervals: skull + hyoid, mandible, molar teeth, incisor teeth, vertebra, humerus, ulna + radius, dorsal rib, ventral rib, sternum + clavicle, scapula, femur, tibia + fibula, ossa coxae and pes + manus.

Major Findings:

In both the growing (10 weeks on F) and the mature rat (52 weeks on F), different bones varied significantly in their capacity to incorporate fluoride. For example, in the mature rat receiving fluoride for 52 weeks, the sternum + clavicle ash contained 0.279 percent F whereas the ventral rib ash contained 0.171% F. In the growing animal fluoride was lost

from the various bones at different rates during the post-fluoride periods. In post-fluoride periods some bones increased in fluoride content indicating a re-deposition of fluoride. The net post-fluoride loss of fluoride for all calcified tissues in the growing and mature rat was 7.5 percent and 11.5 percent respectively.

Significance to Dental Research:

The data indicate that different bones of the body vary in their retention and metabolism of fluoride.

Proposed Course of Project:

Data obtained in 1958 indicate that fluoride markedly lowered the citrate content of bones. This observation will be studied as a basic problem in bone fluoride metabolism.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Zipkin, I., Likins, R. C., and McClure, F. J.: Deposition of Fluoride, Calcium and Phosphorus in Experimental Low Phosphorus Rickets. J. Nutrition 67:59-68, Jan. 1959.

Serial No. NIDR-[illegible]
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Inorganic Chemistry of Dental Tissues, Surface Enamel Chemistry as Affected by Diet and Oral Environment, and their Relation to Dental Caries.

Principal Investigator: H. G. McCann

Other Investigators: None

Man Years (calendar year 1959):

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives:

1. To study properties and structure of mineralized tissue and their reactions with fluoride, phosphate, and calcium.

2. To study the inorganic composition of teeth as influenced by diet or other factors.

3. To determine how the mechanism of calcification is affected by diet.

4. To evolve methods of analysis suitable for the inorganic constituents of mineralized tissues.

5. To develop methods for study of Sr vs. Ca metabolism.

Methods Employed:

1. The teeth and bones of rats receiving diets having variable Ca:P and Mg:Ca:P ratios were analyzed. A new micro method for magnesium was adapted for use with rat enamel.

2. The composition and incorporation of radioactive P^{32} in the enamel of rats fed various mineral-supplemented diets

was studied.

3. The effect of various treatments of rat femurs on the exchange with P^{32} was investigated and the data supplemented by comparative studies on synthetic hydroxyapatite and its fluoride exchange.

4. A method was developed for the estimation of small amounts of Sr in the presence of large amounts of Ca, suited to a study of Ca:Sr metabolism in humans.

5. A preliminary Ca:Sr balance study was carried out on two human adult males.

Major Findings:

1. Further experiments with a skim milk powder diet showed that rats fed this diet with Na_2HPO_4 added have a significantly reduced caries experience. In the teeth and bones of these rats there was a tendency toward a slight decrease in the Ca:P ratio, an increase in magnesium and a decrease in carbon dioxide. However, it seems unlikely that these are factors associated with the decrease in caries.

2. Addition of $CaCO_3$ to the diet increased the Ca:P ratio, lowered magnesium, and increased carbon dioxide in bones and teeth. A considerable increase in the magnesium content of bones and teeth was obtained with magnesium-supplemented skim-milk-powder diets with varying Ca:P ratios. There was no relation of these changes in teeth and bones to the dental caries experience.

3. Various treatments of rat bone (autoclaving, extracting hot or cold, wet ashing, etc) considerably affected their exchange with radioactive P^{32}. Because of changes in crystal surface composition by these various treatments, fluoride ion exchange gave a much more reliable indication of crystal size and of organic matrix affects that did radioactive P^{32}.

4. A preliminary study of the effect of calcium intake on the excretion of strontium was carried out on two adult males. Although the calcium intake was increased four times (0.5 grams per day to 2.0 grams) strontium excretion was unaffected. In both cases almost the entire amount of

strontium ingested (15 mg. per day plus 2-3 mg. of dietary Sr) was excreted--chiefly in the feces. Further studies should be with children whose bones are undergoing calcification.

5. An analytical method for the determination of magnesium in mineralized tissues was perfected. A method for determination of small amounts of strontium in presence of large quantities of calcium by isotope dilution was found to work satisfactorily on samples of urine and feces.

Significance to Dental Research:

As more is learned about the effect of dietary factors on oral tooth surfaces and their concomitant influence on dental caries, less empirical methods may be employed for the prevention of this disease.

Proposed Course of Project:

Project discontinued because of Mr. McCann's transfer.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

McCann, H. G.: Determination of Microgram Quantities of Magnesium in Mineralized Tissues. Anal. Chem. 31:2091-2, Dec. 1959.

McClure, F. J. and McCann, H. G. Dental Caries and the Composition of Bone and Teeth of White Rats, as Affected by Variations in Dietary Ash, Calcium and Phosphorus. Submitted to Archives of Oral Biology.

Serial No. NIDR-30
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Protein and Amino Acid Chemistry. I. Proteolytic Enzymes; Chemical and Molecular Studies, Isolation, Purification and Kinetics. II. Blood Clotting; Enzymology, Metabolism and Molecular Mechanism.

Principal Investigator: Dr. J. E. Folk

Other Investigators: None

Cooperating Units: Drs. K. Laki, J. A. Gladner, Y. Levin, and W. Carroll (NIAMD, Lab. of Physical Biology-36), Dr. E. C. Wolff and Dr. K. A. Piez (NIDR, Lab. of Biochem.)

Man Years (calendar year 1959):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

I. (a) Isolation, molecular and chemical characterization, activation and specificity of carboxypeptidase B, and its use in protein primary-structural-analysis.

(b) The mechanism by which certain metal ions combine with the carboxypeptidases to form more active derivatives.

(c) Development of a simplified procedure for the purification of aminopeptidases.

II. A study of these aspects in the thrombin catalyzed conversion of fibrinogen to fibrin.

(a) Mode of action and specificity of thrombin.

(b) The structural and physiological properties of the peptides formed during conversion.

(c) [illegible] of this [illegible] [illegible] [illegible]

III. Investigations on the intermediate metabolism of fibrinogen.

Methods Employed:

I. (a) Steps in the purification of carboxypeptidase B have been followed by a new and convenient spectrophotometric assay procedure. Physical and chemical procedures include ultracentrifugation, diffusion and electrophoresis, amino acid composition, end-group analysis and metal content.

(b) Studies on the mechanism and specificity of the activation of carboxypeptidases A and B by certain metal ions, principally Co^{++} include reversal of activating effect by dilution of solutions or removal of metal ions, stability of metal-enzyme complexes, and effect of competitive inhibitors.

(c) Ion exchange cellulose chromatography of leucine aminopeptidase has been applied as a method of rapidly purifying this enzyme.

II. (a) Amino acid sequence studies of peptides A and B of cofibrin as described in II (b) are being applied in an effort to gain some insight into the mode of action and specificity of thrombin. Synthetic peptide derivatives containing arginine are prepared and tested as substrates for thrombin

(b) Cleavage by trypsin and chymotrypsin followed by chemical end-group methods have been applied to the structure determination of peptide A and peptide B.

(c) See III.

III. Preliminary studies into a possible system for studying the intermediate metabolism of fibrinogen have been initiated in this laboratory. At present rabbit fibrinogen is being isolated and peptides of the cofibrin investigated in an effort to find a laboratory animal suitable for metabolism studies.

Major Findings:

I. (a) Active carboxypeptidase B has been isolated in purified form from extracts of an acetone powder of autolyzed swine pancreatic glands. Physical and chemical studies on this

highly purified enzyme [illegible] a molecular weight in the vicinity of 35,000, a value very close to that of carboxypeptidase A, an amino acid analysis quite similar to the A enzyme and like the A enzyme one gram atom of zinc per mole of enzyme.

(b) It has been observed that the amidase activity of both carboxypeptidase A and B is increased 100 percent by preincubation with Co^{++} ions. Studies have shown that this activation is dependent upon ion concentration, enzyme concentration, pH, temperature etc. and that this reaction is readily reversible. The native zinc of the enzymes is not replaced by Co^{++} ions under the experimental conditions.

(c) Ion exchange cellulose has offered an excellent and rapid method of purification of leucine aminopeptidase of kidney.

what?

II. (a,b,c) The complete amino acid sequence of peptide A of co-fibrin has been elucidated

III. It has been observed that although the peptides of rabbit co-fibrin differ from those of the bovine system, they may offer an advantage for fibrinogen studies, by containing a tyrosine-o-sulfate grouping.

Significance to Dental Research:

I. A clear evaluation of the specificity and mode of action of carboxypeptidase B contributes knowledge to basic protein metabolism. Carboxypeptidase B has proven to be a valuable tool in both natural and chemically-modified protein-structure research and it complements the classic carboxypeptidase A in this respect.

II. A thorough understanding of the structures and mechanisms involved in the fibrinogen to fibrin reaction should open the way for a more systematic pharmacological and clinical approach to the correction of certain abnormal blood clotting reactions.

Proposed Course of Project:

I. (a) Further physio-chemical studies of carboxypeptidase and attempts to isolate and purify the bovine enzyme. Isolation and purification of the zymogens of these enzymes is contemplated.

(b) Further studies on the mode of activation of carboxypeptidase A by metal ions.

II. (a,b,c) Studies of the amino acid sequence of peptide B of co-fibrin will be continued.

III. The possible incorporation of tyrosine-sulfate into the co-fibrin fraction of rabbit fibrinogen will be studied employing radioactive tracers. In a similar manner, the mechanism of fibrinogen synthesis and breakdown will be investigated.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Gladner, J. A., Folk, J. E., Laki, K. and Carroll, W. R. The Thrombin Induced Formation of Co-fibrin. I. Isolation, Purification and Characterization of Co-fibrin. J. Biol. Chem. 234:62-66, Jan. 1959.

Folk, J. E., Gladner, J. A., and Laki, K. The Thrombin Induced Formation of Co-fibrin. II. Preliminary Amino Acid Sequence Studies on Peptides A and B. J. Biol. Chem. 234:67-70, Jan. 1959.

Folk, J. E., Gladner, J. A. and Levin, Y. The Thrombin Induced Formation of Co-fibrin. III. Acid Degradation Studies and Summary of Sequential Evidence of Peptide A. J. Biol. Chem. 234 2317-2320, Sept. 1959.

Folk, J. E. and Gladner, J. A. Carboxypeptidase B. III. The Specific Esterase Activity. Biochim. et Biophys. Acta 33:570-572, July 1959.

Folk, J. E., Gladner, J. A. and Viswanatha, T. A Simplified Chromatographic Purification of Leucine Aminopeptidase. Biochim. et Biophys. Acta 36:256-257, Nov. 1959.

Folk, J. E. and Gladner, J. A. Cobalt Activation of Carboxypeptidase A J. Biol. Chem. In press.

Serial No. NIDR-31
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Physical Chemistry of Proteins.

Principal Investigator: Dr. Marc Lewis

Other Investigators: Dr. Karl Piez (NIDR)

Cooperating Units: Dr. Harry Saroff (NIAMD-36)

Man Years (calendar year 1959):

Total:	1/4
Professional:	1/4
Other:	none

Project Description:

Objectives:

1. To study the association of calcium, strontium, chloride, and hydroxide ions with human serum albumin.

2. To study the physical characteristics of collagen with reference to structure and function.

Methods Employed:

1. The association of calcium, strontium and chloride ions with human serum albumin was determined by measurement of the free ionic species. Hydroxyl ion association was calculated from pH data.

2. Sedimentation data on parent gelatins from calf-skin collagen were obtained by ultracentrifugation, measurement of the resultant plates, and appropriate calculations.

Major Findings:

Association constants for the binding of calcium, chloride, and hydroxide to human serum albumin have been obtained.

Certain structural relationships in the protein molecule have been postulated to account for these results.

Significance to Dental Research:

1. The association of calcium and strontium with blood proteins is important in the metabolism of these ions.

2. Collagen is a major structural entity in teeth, bone, and periodontium, and probably plays a significant role in the normal function and pathology of these tissues. Basic studies of this protein afford a better understanding of normal and abnormal activity in these tissues.

Proposed Course of Project:

1. The work on the binding of calcium, chloride, and hydroxyl ions to human serum albumin is complete and has been submitted for publication. Work is currently in progress on strontium binding, and, when completed and published, this aspect of the project will be terminated.

2. Studies on physical properties of collagens will include collagens from different sources. Amino acid composition will be related to structure and function.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Lewis, M. and Sollner, K. Preparation and Properties of Improved Protamine Collodion Matrix Membranes of Extreme Ionic Selectivity. J. Electrochem. Soc. 106:347-354, April 1959.

Serial No. NIDR-12
1. Biochemistry
3. Bethesda, Md

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemistry of Salivary Proteins

Principal Investigator: Dr. Marc S. Lewis

Other Investigators: Dr. F. J. McClure, Dr. I. Zipkin

Cooperating Units: None

Man Years (calendar year 1959):

Total: 3/4
Professional: 3/4
Other: none

Project Description:

Objectives:

1. To fractionate and isolate salivary proteins and characterize them physically and chemically.

2. To attempt correlation of these properties to oral and systemic pathology generally and to the normal and abnormal physiology of the salivary glands.

Methods Employed:

Samples of saliva were obtained from rats under varied experimental conditions, suitably prepared, and analysed by moving-boundary electrophoresis (Tiselius method). The mobilities and relative distribution of the protein components were determined by analysis of the photographic records of the Schlieren patterns. The following studies on rat saliva were performed: effect of maturation on Sprague-Dawley (S-D) rats; effect of diet on S-D rats; effect of stress on Osborne-Mendel (O-M) rats; and comparison of S-D, O-M, and wild rats.

Major Findings:

Seven discrete components have been found in all saliva samples analysed. Their electrophoretic mobilities in pH 8.6 veronal buffer ($u = 0.1$) did not vary significantly. Differences in the quantitative distribution of the salivary proteins were found between the S-D, O-M and wild strains of rats. Significant changes in distribution occurred with the maturation of S-D rats. Marked changes in distribution were found when young, sexually mature S-D rats were placed on a protein-free diet. These changes were less marked in older rats, and were reversed by returning the animals to a normal diet. These diet-induced changes are possibly an effect of depletion of body protein. The S-D rats on a high-protein diet and the O-M rats which were subjected to stress which resulted in abnormal reproductive behavior did not exhibit any significant deviations in salivary protein distribution patterns.

Significance to Dental Research:

This information contributes to a better understanding of saliva biochemistry and salivary gland physiology.

Proposed Course of Project:

A detailed analysis of the electrophoretic patterns, and a histological study are planned to ascertain the effect of the low protein diet on the salivary glands. This project will be terminated following preparation of this material for publication.

Part B included No

Serial No. NIDR-32
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemical and Biophysical Studies of Calcification.

Principal Investigator: Dr. R. C. Likins

Other Investigators: Dr. A. S. Posner and H. G. McCann

Cooperating Units: National Bureau of Standards (Dr. A. S. Posner)

Man Years (calendar year 1959):

Total:	2 3/4
Professional:	1
Other:	1 3/4

Project Description:

Subproject A: (With Dr. A. S. Posner and H. G. McCann)

Objectives:

To investigate the skeletal metabolism of calcium and strontium.

Methods Employed:

Samples of calcium hydroxyapatite were prepared by precipitation from solutions containing radioactive calcium and strontium. The method of synthesis was varied to yield precipitates with different mean crystal sizes. Estimates of crystal size were obtained by either X-ray diffraction or electron microscopy. Exchange studies were carried out with these materials to investigate the comparative release of the isotopes in stable calcium and strontium solutions.

Major Findings:

1. Strontium did not substitute freely for the calcium of hydroxyapatite.

2. The studies showed a discrimination against strontium in crystal formation which increased with crystal perfection.

Significance to Dental Research:

Results which have been obtained *in vivo*, i.e. a decrease in the Sr^{89}:Ca^{45} ratio of bone with time, may be due, in part, to a discrimination against strontium during crystal formation and during crystal perfection through recrystallization.

Proposed Course of Project:

This work has been completed and the report submitted for publication.

Subproject B:

Objectives:

To investigate the renal excretion of calcium and strontium.

Methods Employed:

Radioactive strontium and/or calcium was administered to rats and the urinary excretion of these ions was studied.

Major Findings:

Double tracer studies performed in this laboratory (see Project Report for Calendar Year 1958) have shown that the comparative renal clearance of calcium-45 and strontium-89 is presumably affected by the nutritional status of the aminal. The comparative renal clearance of calcium-45 and stable calcium is different in starved and non-starved rats. For example, during two successive 30-minute periods following radiocalcium injection, rats which had been starved for 12 hours excreted the same amount of total calcium as their controls, but only one tenth to one fourtieth as much calcium-45. The concentration of radiocalcium in the plasma was the same in both groups of rats 30 minutes and one hour after injection.

Significance to Dental Research:

This research will provide further information on mineral metabolism.

Proposed Course of Project:

While the relative amounts of free and protein-bound Ca^{45} or Sr^{89} appear to be unaffected by inanition, possible differences in the binding of these ions by smaller molecules is being investigated by membrane dialysis techniques.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Likins, R. C., Scow, R. O., Zipkin, I. and Steere, A. C. The Deposition and Retention of Fluoride and Radiocalcium in the Growing Rat. Am. J. Physiol. 197:75-80, July 1959.

Likins, R. C., Posner, A. S., Kunde, M. L. and Craven, D. L. Comparative Metabolism of Calcium and Strontium in the Rat. Arch. Biochem. Biophys. 83:472-481, Aug. 1959.

Likins, R. C., Piez, K. A. and Kunde, M. L. The Mineralizing Leg Tendons of the Turkey. I. The Nature of the Protein and Mineral Phase. Accepted for AAAS Monograph.

Piez, K. A. and Likins, R. C. The Nature of Collagen, Part II. The Vertebrate Collagens. Accepted for AAAS Monograph.

Serial No. NIDR-34
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Analytical and Structural Aspects of Collagen in Relation to Function and Disease.

Principal Investigator: Dr. K. A. Piez

Other Investigators: Dr. R. C. Likins, Dr. E. Weiss

Cooperating Units: None

Man Years (calendar year 1959):

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

1. It is the long-range purpose of this project to examine collagens from various sources with respect to their chemical and physical nature (such as amino acid composition, amino acid sequence, crosslinking and solubility) in an attempt to correlate these factors with function of the tissue or with disease states.

2. The immediate objectives are (a) to compare the amino acid composition of collagens from different tissues and different animals, and (b) to study the structure of the collagen molecule in relation to its amino acid composition.

Methods Employed:

Usual laboratory procedures.

Major Findings:

1. Amino acid analyses have been performed on a number of

collagens from invertebrate phyla. These resemble vertebrate collagen in that they contain hydroxyproline and hydroxylysine and one-third of the residues are glycine. In other respects they are quite different. The similarities are in accord with the requirements of the structure of the molecule. The differences may be related to function or structure at the fibril or tissue level.

2. The thermal denaturation of collagen has been thought to be a function of the hydroxyproline content and related to hydrogen bonding by the hydroxy group of hydroxyproline. We have now shown that this is probably not correct. Analysis of data of five fish collagens, studied by us, and data from the literature, indicate that thermal denaturation is associated with the total content of pyrrolidine rings (proline plus hydroxyproline). This is in accord with the suggestion that the pyrrolidine rings have a strong directive influence on the structure of polypeptide chains of the type characteristic of collagen. This evidence also allows conclusions to be drawn with regard to other aspects of the molecular structure of collagen.

3. It has been shown that chemical or thermal denaturation of collagen results in the formation of two fragments from the original collagen molecule. Chemical and physical data indicate that these two fragments differ in amino acid composition and in molecular weight. The collagen molecule is apparently made up of two of one and one of the other fragments.

Significance to Dental Research:

Collagen is the major protein of skin (dermis), tendon, bones, and teeth (dentin). It may play a role in the function of these tissues and in pathological states which involve them, including dental caries and periodontal disease. Basic studies of this protein may, therefore, contribute to a better understanding of function and disease.

Proposed Course of Project:

Studies are continuing on invertebrate collagens with emphasis on matrices of calcified tissues. It is proposed to obtain more collagens of epithelial origin and study their chemical properties. The denaturation of collagen will be examined further in the hope of obtaining basic information on the structure of this protein.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Piez, K. A. and Gross, J. The Amino Acid Composition and Morphology of Some Invertebrate and Vertebrate Collagens. Biochim. et Biophys. Acta 34:24-39, July 1959.

Gross, J. and Piez, K. A. Calcification in Biological Systems, AAAS Symposium, Chap. 17I, The Nature of Collagen. I. The Invertebrate Collagens. In press.

Piez, K. A. and Gross, J. Calcification in Biological Systems, AAAS Symposium, Chap. 17 II, The Nature of Collagen. II. The Vertebrate Collagens. In press.

Likins, R. C., Piez, K. A., and Kunde, M. L. Calcification in Biological Systems, AAAS Symposium, Chap. 5 III. Mineralization of the Turkey Leg Tendon. III. Chemical Nature of the Protein and Mineral Phases. In press.

Piez, K. A. The Relation Between Amino Acid Composition and Denaturation of Vertebrate Collagens. J. Am. Chem. Soc. In press.

Piez, K. A. and Gross, J. The Amino Acid Composition of Some Fish Collagens: The Relation Between Composition and Structure. J. Biol. Chem. In press.

Serial No. NIDR-35
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biochemistry of Amino Acids and Proteins in Mammalian Cell Cultures.

Principal Investigator: Dr. K. A. Piez

Other Investigators: None

Cooperating Units: Dr. H. Eagle (NIAID-50)

Man Years (Calendar year 1959):

Total:	1 1/2
Professional:	1/2
Other:	1

Project Description:

Objectives:

To obtain basic information on protein and amino acid metabolism in mammalian cells in culture. Current studies relate to protein synthesis and cystine binding by serum proteins.

Methods Employed:

Usual laboratory procedures.

Major Findings:

1. It has been found that intracellular protein turnover proceeds at a rate of about 1 percent per hour. This represents an average half-life of the cellular protein of about 3 days. This is of considerable importance in any consideration of the mechanism of protein synthesis since the process must be easily reversible by either the same or a different pathway.

2. Whether cells in culture utilize protein in the medium is a question of importance in any study of protein or amino

acid metabolism. We have approached this problem by employing labeled serum protein. It was found that 3 to 6 percent of the cell protein derived from protein in the medium. This is considered to be a maximum and owing primarily to the possibility of mechanical mixing.

3. Cystine is an essential amino acid for cells in culture although under some conditions growth will continue slowly in the absence of added cystine. We have shown that binding of cystine to serum protein occurs through disulfide bridges during storage and that this is released during growth of cells. Therefore, for studies involving cystine metabolism it is important to use fresh serum dialyzed or otherwise desalted before cystine binding can occur.

Significance to Dental Research:

Cell culture is a relatively new tool suitable for the study of many metabolic processes. These basic studies are important to all branches of the medical sciences. Topics more immediately related to dental research, such as collagen biosynthesis, are amenable to study with these techniques.

Proposed Course of Project:

It is planned to continue these studies along the lines already started. Experiments are underway involving cystine biosynthesis, amino acid transport, and collagen biosynthesis in cell culture.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards and Publications

Publications other than abstracts from this project:

Eagle, H., Piez, K. A., Fleischman, R. and Oyama, V. I. Protein Turnover in Mammalian Cell Cultures. J. Biol. Chem. 234:592-597, March 1959.

Eagle, H., Oyama, V. I. and Piez, K. A. The Reversible Binding of Cyst(e)ine by Serum Protein, and its Bearing on the Cyst(e)ine Requirement of Cultured Mammalian Cells. J. Biol. Chem. In press.

Eagle, H. and Piez, K. A. The Utilization of Proteins by Cultured Human Cells. J. Biol. Chem. In press.

Serial No. NIDR-36
1. Biochemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Role of Tetracycline in Experimental Caries in the White Rat.

Principal Investigator: Dr. R. H. Larson

Other Investigators: Dr. I. Zipkin

Cooperating Units: None

Man Years (Calendar year 1959):

Total:	2 1/4
Professional:	1 1/4
Other:	1

Project Description:

Subproject A:

The Effect of Tetracycline on Caries Activity.

Objectives:

To study the relation of tetracycline to dental caries in the offspring, when it is given to the mother rat at various periods during pregnancy and/or during lactation and to the offspring immediately following weaning.

Methods Employed:

Pregnant female rats were given tetracycline in the drinking water (a) during pregnancy only, (b) during pregnancy and the first two weeks of lactation, (c) during pregnancy and until the offspring were weaned at 3 weeks of age, (d) for the last 7 days before weaning and (e) for the first 7 days on a caries producing diet. The offspring were housed two littermates per cage and were given a caries-test diet for 60 days, after which the teeth were examined for caries and

compared with control rats.

Major Findings:

It was shown that tetracycline given under all of the conditions mentioned above, caused a highly significant reduction in the development of caries in the offspring and also had no detrimental effect on the mother or the offspring.

Significance to Dental Research:

These findings demonstrate again the role of microorganisms in caries etiology and indicate that some protective anticaries "factor" may be acquired prior to exposure to a caries producing diet.

Subproject B:

Objectives:

The relation of pretreatment with tetracycline, to the transmission of dental caries in white rats.

Methods Employed:

Rats which were the offspring of Group "B" (tetracycline given to the mothers during pregnancy and the first 2 weeks of lactation) and the offspring of Group "E" (tetracycline to mother and pups during the last 7 days prior to weaning) were housed as follows: (a) two littermates from the same group per cage, (b) one "B" or one "E" rat and one control rat per cage and (c) two control rats per cage.

Major Findings:

The rats which had been subjected to a pretreatment with tetracycline and housed separately, showed minimal caries development. The animals pretreated with tetracycline and housed in a cage with untreated control animals developed extensive caries, comparable to that of their caries susceptible cage-mates, and caries susceptible control animals housed two per cage. These findings suggest that tetracycline treatment was an effective means of reducing caries activity in the rat, but only when the animals were

not in contact with caries susceptible rats.

Significance to Dental Research:

These findings provide information on the influence of prenatal and lactation factors and post weaning environment on the development of dental caries in white rats.

Proposed Course of Project:

Since tetracycline is well tolerated by the white rat, it provides a means of studying the cariogenic microbial environment in this species. (1) Different methods of inoculation and inoculation with specific organisms may be studied. (2) Pretreatment with tetracycline, followed by subjection to a common inoculum, can provide a means by which the oral flora may be partially controlled in experimental caries studies.

Part B included Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Larson, R. H. The Effect of EDTA on the Pattern of Caries Development and its Association with Biologic Changes in the Rat. J. Dent. Res. 38:1207-1212, Nov.-Dec. 1959.

Zipkin, I., and Larson, R. H. Caries Potentiating Effect of Na EDTA, Ca EDTA and Mg EDTA in the Rat. J. Dent. Res. 38: 1240, Nov. - Dec. 1959.

Serial No. NIDR-37
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Metabolism of Oral Flora and Salivary Glands.

Principal Investigator: Subproject A: Dr. H. Blumenthal;
Subproject B: Dr. H. Blumenthal and
Dr. T. Shiota

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):

Total:	3
Professional:	1 1/2
Other:	1 1/2

Project Description:

Subproject A:

Nucleoside Metabolism in Rat Submaxillary Glands.

Objectives:

To elucidate the source and mode of ammonia release by rat submaxillary gland preparations incubated in the presence of certain purine derivatives.

Methods Employed:

Standard methods for (a) the extraction of tissues, (b) ion exchange and paper chromatography, (c) analysis for metabolites, and (d) spectrophotometry.

Major Findings:

In some experiments using rat submaxillary glands, ATP alone catalyzed the release of ammonia. Alanine and glutamic acid were ruled out as a source of ammonia by direct assay and

chromatography for keto acids, as well as through assay for ammonia release. Since ATP seemed the prime source of ammonia, the question of whether ATP *per se* or one of its breakdown products was releasing ammonia was investigated. The results indicate the presence of adenosine triphosphatase, a pyrophosphatase and adenosine deaminase. Such systems have been reported in other tissues. Two other naturally-occurring amino-group-containing nucleic acid bases, cytosine and its nucleoside, were inactive in the release of ammonia. Guanine and guanosine were active while guanylic acid was not. Guanine activity, however, was negligible when compared to guanosine.

Significance to Dental Research:

Basic data are being obtained relative to the metabolism of salivary hexosamines.

Proposed Course of Project:

Further studies on the purification, characterization and the exact mechanism of guanosine deamination in submaxillary glands.

Subproject B:

Hexosamine Metabolism of Microorganisms.

Objectives:

To study the metabolism of hexosamines by microorganisms, in terms of metabolic breakdown and utilization.

Methods Employed:

1. Standard procedures for the study of growth of bacteria and isolation of enzyme systems from microorganisms.

2. Analytical procedures for metabolites.

3. Ion exchange and paper chromatography, and spectrophotometric analyses.

Major Findings:

Lactobacillus casei was able to grow in a media containing

glucosamine or galactosamine as the sole carbohydrate. The growth characteristics of this organism in such a media were studied. Washed, resting cell, suspensions of *L. Casei* grown under varying conditions were incubated with the hexosamines or glucose as substrates. Analyses for ammonia liberation; ketohexose formation and lactic acid production indicate that these cells utilize the hexosamines as a source of energy and carbon, and that ammonia and lactic acid are produced.

Significance to Dental Research:

Hexosamines are constituents of salivary polysaccharides and as such are present in the oral cavity. The mode of attack of oral microorganisms on constituents of the saliva may be significant in the etiology of oral diseases.

Proposed Course of Project:

We are continuing to screen lactobacilli for an organism which is more active than the *L. casei*. We are also working on a media that would offer a more economical source of galactosamine for use in large batch-growth purposes. From such cells we will isolate and purify the enzymatic systems concerned with hexosamine dissimilation. In addition, the synthesis of a number of intermediary compounds is indicated. Thus, we propose to elucidate basic hexosamine metabolism.

Part B included No

Serial No. NIDR-16
1. Biochemistry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Collagen. I. Biochemical Studies on Collagenolysis. II. Relation of Amino Acid Composition and Reactivity to Polymer Form.

Principal Investigators: Dr. George R. Martin and Dr. S. E. Mergenhagen.

Other Investigators: None

Cooperating Units: American Dental Association

Man Years (Calendar year 1959):

Total:	1 1/4
Professional:	1 1/4
Other:	none

Project Description:

Objectives:

1. To study the events leading to and causing the breakdown and removal of tissue collagen in normal and pathological conditions.

2. To gain information concerning the key chemical groups that determine the structure of the macromolecular collagen aggregate.

Methods Employed:

Usual laboratory procedures.

Major Findings:

1. The normal urinary excretion of bound and free hydroxyproline has been measured in guinea pigs and rabbits. If the rate of collagenolysis is increased by feeding guinea pigs a vitamin C deficient diet, it does not induce the

increased excretion of hydroxyproline in the urine. Intradermal collagenase injection releases large amounts of collagen from the skin of rabbits and guinea pigs but does not increase the urinary excretion of hydroxyproline. These findings suggest that the body contains active systems for disposing of collagen and the products of collagen degradation.

2. Collagen fails to assume the characteristic 640 Å repetitive structure when gelled at a pH of less than 6.5. Fibers precipitating from solutions having low pH values are more easily solubilized than normal fibers. Analysis of the titration curves of fibers gelled at various pH's indicates that the imidazole group of histidine is not available for reaction with hydrogen ion in collagen with a periodicity of 640 A and is reactive in unbanded collagen. These results suggest that the degree to which imidazole residues are ionized is an important factor in determining polymer form and thus the physical properties of collagen.

Significance to Dental Research:

Collagen is an important constituent of the hard tissues. A clarification of the events involved in its formation and destruction may therefore contribute to our understanding of pathological conditions where collagen metabolism may be altered.

Proposed Course of Project:

1. It is planned to examine the ability of various mammalian tissues and enzyme systems to metabolize collagen.

2. Further characterizations of the groups influencing and determining the structure of the collagen aggregate are in progress.

3. The structure which collagen assumes upon polymerization is dependent upon the milieu in which gelatin occurs. It is planned to use this fact to further elucidate the structure of collagen.

Part B included No

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1959

EPIDEMIOLOGY & BIOMETRY BRANCH:

39. A. L. Russell: The Epidemiology of Dental Caries - Including the Fluoride-Dental Caries Relationship.

40. A. L. Russell and Ernest C. Leatherwood: Epidemiology of Oral Diseases in Vietnam.

41. A. L. Russell and C. L. White: Relation Between Dental Caries and Periodontal Diseases.

42. A. L. Russell and C. L. White: Epidemiology of Oral Diseases among Alaskan Natives.

43. C. J. Donnelly and N. W. Littleton: Epidemiology of Fractured Incisors.

44. Ernest C. Leatherwood and J. C. Greene: Epidemiology of Oral Diseases in Ecuador.

45. C. J. Donnelly: Study of Periodontal Disease and Caries in Adventists Families.

46. N. W. Littleton: Epidemiology of Oral Diseases among Ethiopians.

Serial No. NIDR-11
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Epidemiology of Dental Caries - Including the Fluoride - Dental Caries Relationship

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. C. L. White

Cooperating Units: Board of Education, City of Grand Rapids; City-County Health Department, Grand Rapids, Michigan; Michigan Department of Health; Montgomery and Prince Georges Counties, Maryland

Man Years (calendar year 1959):
Total: 3
Professional: 1-1/4
Other: 1-3/4

Project Description:

Objectives:

1. To observe the effects of fluoridation of a community water supply on children whose initial exposure to fluoride occurred at different age levels.

2. To define the epidemiological characteristics of dental caries.

3. To obtain basic information concerning the nature of dental caries and the dental tissues susceptible to attack so that present methods of control may be perfected and, possibly, new methods may be evolved.

Methods Employed:

Direct examination of population groups with subsequent analysis of findings to determine relative risks under varying conditions

Major Findings:

1. Progressive reduction in the prevalence of dental caries in the Grand Rapids population under study continued during the past year. Sodium fluosilicate, a less expensive compound, appears as effective as sodium fluoride when used in fluoridation of public water supplies

2. After seven years of fluoridation in Montgomery and Prince Georges Counties, Maryland, there has been a significant reduction in dental caries. From children up through age nine whose teeth largely developed during the use of fluoridated water, the curve for DMF means closely approximates an expectancy curve obtained from an area whose population uses a water containing natural fluorides. Two significant aspects of the fluoride - dental caries hypotheses appear to be:

   a. Time available for the accumulation of fluoride in a tooth prior to eruption.

   b. Time at risk, or the length of time a tooth is exposed to attack by dental caries.

Significance to Dental Research:

Findings from this study are expected to expand present knowledge of the beneficial effects of controlled fluoride ingestion on the dentition. Through detailed analysis, an attempt will be made to secure information on the action of fluoride during the various formative periods of the teeth. Inferences can be made on the duration of the inhibitory effects of fluoride on dental caries.

Proposed Course of Project:

Studies on the epidemiology of dental caries will continue. Concentrated effort will be made in areas where basic information concerning the action of fluoride in humans is equivocal or entirely lacking. Examination of these population samples will continue until:

1. The full inhibitory effect of fluoride on dental caries becomes evident in each age group and inferences can be made as to the duration of this effect.

2. The prevalence of fluorosis has reached a maximum.

3. Information is available on the modes of action of fluoride on the dental tissues.

Part B included: Yes

Serial No. NIDR-1

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, Albert L. and White, Carl L., Dental Caries in Maryland children after five years of fluoridation. Public Health Reports, 74:289-95, April 1959.

Honors and Awards relating to this project:

None

Serial No. NIDR-40
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiology of Oral Diseases in Vietnam.

Principal Investigators: Dr. A. L. Russell and
Dr. Ernest C. Leatherwood

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense.

Man Years (calendar year 1959):

Total:	3/4
Professional:	3/4
Other:	0

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Vietnam and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risk under varying conditions.

Major Findings:

Field examinations in progress.

Significance to Dental Research:

This project presents an opportunity to expand present information on the epidemiological characteristics of oral diseases. Furthermore, possible associations may be established between the occurrence of oral diseases and environmental, medical, biochemical, and nutritional factors.

Proposed Course of Project:

Tabulation and analysis of the data obtained, and correlation of findings with those from other surveys in an attempt to improve present knowledge concerning the occurrence of oral diseases in various population groups.

Part B included: No

Serial No. [illegible]
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Relation Between Dental Caries and Periodontal Diseases.

Principal Investigator: Dr. A. L. Russell

Other Investigators: Mr. Carl L. White

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1
Professional: 1/2
Other: 1/2

Project Description:

Objectives:

To study possible correlations between dental caries, fluoride, and periodontal diseases.

Methods Employed:

Analysis of data obtained from studies on caries and periodontal diseases conducted by the Epidemiology and Biometry Branch.

Major Findings:

1. Preliminary findings indicate that there is no significant difference in the occurence of periodontal diseases about teeth with or without dental caries.

2. Periodontal tissues are not harmed by use of a fluoride-bearing domestic water.

Significance to Dental Research:

This project expands the program of the Epidemiology and Biometry Branch through statistical investigation of data available from other projects.

Proposed Course of Project:

To continue to expand the study as outlined.

Part B included: Yes

Serial No. NIDR-41

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Russell, Albert L., and White, Carl L., Fluorides and Periodontal Health. pp. 115-27. (In: Muhler and Hine, editors, Fluorine and dental health; the pharmacology and toxocology of fluorine. Bloomington, Indiana University Press, 1959. 216 p.)

Honors and Awards relating to this project:

None

Serial No. NIDR-62
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiology of Oral Diseases among Alaskan Natives

Principal Investigator: Dr. A. L. Russell

Other Investigator: Mr. C. L. White

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense

Man Years (calendar year 1959):
Total: 1-3/4
Professional: 1/2
Other: 1-1/4

Project Description:

Objectives:

To determine the prevalence of dental caries, periodontal diseases, and malocclusion among Alaskan natives and to investigate possible relationships of these disorders to certain nutritional and geographic characteristics of this group.

Methods Employed:

Direct examination of members of the Alaskan National Guard. Subsequently, statistical analysis of data obtained to determine relative risks under varying conditions.

Major Findings:

Dental examinations of approximately 700 Eskimo men of the Alaskan National Guard revealed significant information concerning oral health in this population group. Preliminary analysis of data indicated interesting differences when findings from "primitive" groups were compared with those from groups living under more civilized conditions. Dental caries was virtually non-existant in individuals from remote villages.

Many of the primitive groups were essentially free of periodontal diseases. Other similarly primitive groups exibited a uniformly prevalent, severe gingivitis. Contrary to generally accepted theory, this gingivitis demonstrated little tendency toward progression into chronic destructive periodontal diseases even in the absence of personal oral hygiene or professional dental care. In contrast to these findings in the more "primitive" groups, Eskimos that had lived for some time under relatively "civilized" conditions, demonstrated prevalence and severity of oral diseases quite comparable to those seen in average male population groups within the United States.

Significance to Dental Research:

The spectrum of living conditions within this Eskimo group affords a unique opportunity to study the influence of "civilization" on oral diseases. Findings from the "primitive" groups are relatively uninfluenced by professional dental care. An opportunity is also present for evaluating oral diseases in terms of the various nutritional patterns included within this population sample.

Proposed Course of Study:

Data from this study undoubtedly represents a basis from which suggestions may be formulated for improving the oral health status of the Alaskan peoples. Furthermore, analysis of these data should contribute to our knowledge of the epidemiological characteristics of oral diseases. It is planned to extend this project to include a study of children from "primitive" village schools.

Part B included: Yes

Serial No. NIDR-42

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Interdepartmental Committee on Nutrition for National Defense. Alaska: an appraisal of the health and nutritional status of the Eskimo. Part III, The Dental Study, pp. 33-39. August, 1959.

Honors and Awards relating to this project:

None

Serial No. NIDR-43
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiology of Fractured Incisors

Principal Investigator: Dr. C. J. Donnelly

Other Investigator: Dr. N. W. Littleton

Cooperating Units: None

Man Years (calendar year 1959):

Total	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives:

To determine, by tooth type, the prevalence of fractured permanent incisors and the extent of damage to the dental tissues resulting from accidents.

Methods Employed:

The prevalence of fractured incisors and the extent of damage is being ascertained by direct observation of several school populations including all grades - kindergarten through high school. By interview, information is being obtained concerning the scene, time and circumstances of the accident. The age, sex, predominant hand, presence of restoration, and student's activity at the time of the accident also is being recorded.

Major Findings:

In the populations thus far surveyed, there are many more upper incisors involved than lower, and there appear to be slightly more fractures on the left than the right. The most susceptible tooth in these populations is the upper left central incisor. More fractures are being recorded than reported in some of the earlier literature. Many of the cases in which the fracture extends into the dentin were not restored at the time of examination.

Significance to Dental Research:

Fractured anterior teeth are disturbing to the child and to the parent and often are difficult to restore satisfactorily. It is hoped that knowledge of the circumstances of the accident will permit classification of some as preventible and eventually lead to recommendations for accident prevention.

Proposed Course of Project:

The project will be continued to include some five thousand children from kindergarten through high school.

Part B included: No

Serial No. NIDR-44
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiology of Oral Diseases in Ecuador.

Principal Investigators: Dr. Ernest C. Leatherwood and
Dr. John C. Greene

Other Investigators: None

Cooperating Units: Office of the Chief Dental Officer, USPHS
Interdepartmental Committee on Nutrition
for National Defense.

Man Years (calendar year 1959):
Total: 3/4
Professional: 1/2
Other: 1/4

Project Description:

Objectives:

To determine epidemiological characteristics of oral diseases in a sample population from Ecuador, and to relate these findings to certain environmental, medical, biochemical, and nutritional data in an attempt to establish possible associations.

Methods Employed:

Direct examination of population groups followed by statistical analysis of findings to determine relative risks under varying conditions.

Major Findings:

Tabulation and analysis of data has not progressed sufficiently to allow statements on findings.

Significance to Dental Research:

This project presents an opportunity to expand information on the epidemiological characteristics of oral diseases. Further, possible associations may be established between the occurrence of oral disease and environmental, medical, biochemical, and nutritional factors.

Proposed Course of Project:

Tabulation and analysis of data obtained, and correlation of findings with those from other surveys in an attempt to improve present knowledge concerning the occurrence of oral diseases in various population groups.

Part B included: No

Serial No. [illegible]
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Periodontal Disease and Caries in Adventists Families

Principal Investigator: Dr. C. J. Donnelly

Other Investigators: None

Cooperating Units: Conferences of Seventh Day Adventists in Indiana, North Dakota, Wisconsin, Michigan, Oklahoma, Southern New England, and Maryland

Man Years (calendar year 1959):
Total: 2
Professional: 3/4
Other: 1-1/4

Project Description:

Objectives:

1. To investigate the influence of familial factors and of geographic location on periodontal diseases and dental caries among Adventists families.

2. To determine if the dental caries rate among Adventists children is lower than among comparable non-Adventists children.

3. To study the prevalence and severity of periodontal disease in children and adults from a related group of individuals.

Methods Employed:

Direct examination of parents and children attending Seventh Day Adventists Camp Conferences in different parts of the United States. The data will be treated statistically to determine the influence of familial factors, geographic location and Adventists health reforms.

Major Findings:

Children examined at Adventists Camps show a consistently low caries rate regardless of the part of the country in which they live. No geographic differences were apparent in the periodontal status of the Adventists examined at the seven camps. There is a tendency for the offspring to reflect the periodontal status of their parents.

Significance to Dental Research:

1. The low-caries rate consistently found among Adventists children may be related to the dietary recommendations of the "health reform" suggested by the Adventists Church. Further study might add to our information on the influence of diet on caries rates.

2. By examining Adventists in different parts of the country, some information will be obtained on the influence of geographic location on caries and periodontal disease. By determining if one parent exerts more influence than the other on the periodontal status of the offspring, additional information may be gained about the factors influencing the severity of periodontal disease.

Proposed Course of Study:

1. A controlled study will be carried out to determine the magnitude of the difference in caries rates between comparable groups of Adventists and non-Adventists children.

2. In order to determine the effects of geographic location on caries and periodontal disease, Adventists populations in parts of the country not previously included will be examined.

Part B included: No

Serial No. [illegible]
1. Epidemiology & Biometry
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Epidemiology of Oral Diseases among Ethiopians.

Principal Investigator: Dr. Norman W. Littleton

Other Investigators: None

Cooperating Units: Interdepartmental Committee on Nutrition for National Defense.

Man Years (calendar year 1959):
Total: 1-3/4
Professional: 3/4
Other: 1

Project Description:

Objectives:

1. To determine the oral health status in a random sample of Ethipians; in particular, to determine the prevalence and epidemiological characteristics of dental caries, periodontal diseases, fluorosis, malocclusion and attrition.

2. To relate these findings to medical, nutritional, and environmental factors in an effort to establish possible associations.

Methods Employed:

Direct examination of an Ethiopian sample with subsequent analysis of examination data to determine relative risk under varying conditions.

Major Findings:

Ethiopians subsisting on a high (natural) carbohydrate diet had a very low prevalence of dental caries. However, the DMF rate appeared to be related to sugar consumption. Prevalence and severity of periodontal disease were high.

There were no significant differences in the occurrence of periodontal diseases by sex, ethnic origin, or geographic area. Nutritional factors appeared to exert only a minor influence on the periodontal tissues in individuals from this sample. Positive associations existed between severity of periodontal diseases and the state of oral cleanliness in this group.

Significance to Dental Research:

This project presented an opportunity to study a population in which little was known concerning oral health status, and one in which the effects of personal oral hygiene and professional dental care would be minimal. Findings could be evaluated in terms of certain environmental, medical, biochemical, and nutritional data in an effort to establish possible associations

Proposed Course of Project:

1. To complete the tabulation and analysis of the data.

2. To utilize findings to formulate possible suggestions for improving oral health conditions within Ethiopia.

3. Through correlating these findings with those of other surveys, attempt to expand information concerning the epidemiological characteristics of oral diseases - particularly dental caries and periodontal disease.

Part B included: Yes

Serial No. [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Interdepartmental Committee on Nutrition for National Defense. Preliminary Report: Ethiopia Nutrition Survey. Section IX. Dental Findings. pp. 69-73, June, 1959.

Honors and Awards relating to this project:

None

NATIONAL INSTITUTE OF DENTAL RESEARCH

Individual Project Reports
Calendar Year 1959

CLINICAL INVESTIGATIONS:

47 (c). C.J. Witkop, Jr.: Tri-racial isolates in Eastern United States.

48 (c). C.J. Witkop, Jr.: Hereditary defects of enamel and dentin (Michigan study).

49 (c). C.J. Witkop, Jr.: Genetic study in Southern Maryland.

50 (c). C.J. Witkop, Jr.: Hereditary eye defects.

51 (c). D.L. Rucknagel: Sickle cell anemias and other inherited hematological disorders.

52 (c). J.D. Niswander: Oral aspects of the results of consanguineous marriage in Hiroshima and Nagasaki, Japan.

53 (c). R.M. Stephan: Studies of the oral microbiota and oral environment in relation to dental and systemic diseases.

54. R.M. Stephan: Interrelationships between local and systemic factors in the etiology of dental diseases in rats.

55 (c). A.D. Merritt: Studies on enzymatic reactions of cyclic alcohols and steroids.

56 (c). A.D. Merritt: Studies of the urinary excretion products of histidine in patients with liver disease.

57 (c). P.J. Coccaro: Serial cephalometric study of the longitudinal growth of the soft palate and nasopharynx in cleft palate children.

58 (c). E.J. Driscoll: General anesthesia (dental) baseline data.

59 (c). I.I. Ship: Investigations into the etiology of recurrent aphthous ulcerations and other oral nonmalignant soft tissue diseases.

60 (c). I.I. Ship: Stress in the dental patient.

CLINICAL INVESTIGATIONS: (Continued)

61 (c). I.I. Ship: Secretory sialography in patients with Sjögren's Syndrome and rheumatoid arthritis.

62 (c). I.I. Ship: Effect of dietary phosphate on dental caries in children.

63 (c). P.N. Baer: Studies on periodontal dressings. Part II. A hydrogenated fat-zinc bacitracin dressing.

64. P.N. Baer: Studies on periodontal disease in the mouse.

65 (c). H.R. Stanley, Jr.: Histopathology of the human pulp.

Serial No. NIDR-47 (c)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Tri-racial Isolates in Eastern United States

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: None

Cooperating Units: Calvin L. Beale, Agricultural Marketing Service, U. S. Department of Agriculture.

Man Years (calendar year 1959):
Total: ½
Professional: ½
Other:

Project Description:

Objectives:

1. To determine the number, types, location and size of tri-racial isolates in Eastern United States.

2. To determine the degrees of inbreeding shown by various isolates.

3. To determine the amount and types of hereditary illness shown by the groups.

4. To verify or not the assumption that more individuals will show homozygous recessive hereditary illness in such populations.

5. To investigate the social consequences of isolate formation and their effects on the distribution and prevalence of intrinsic disease.

6. To investigate and compare the prevalence, types and inheritance patterns of intrinsic illness with in-group and out-group marriages, with degrees of consanguinity, with various other tri-racial groups, and with the general population.

7. In summary, the purpose of this investigation is to find, enumerate and describe all tri-racial isolates residing in Eastern United States; to investigate the degree of consanguinity in these groups; to look for unusual types of matings, rare in human material, that are of special value in determining modes of inheritance, gene interaction, and biochemical pathways; to investigate the hereditary diseases present in these groups; and to utilize the patient material found for further clinical and biochemical genetic investigations. It is assumed that in isolate populations, consanguinity will be prevalent; and it is further assumed that an increased number of individuals in such a population will show homozygous recessive illness. It is also assumed that all cases of a specific hereditary illness from any one group will nearly always be due to the same gene defect, thus providing an epidemiological method for selecting a large sample of individuals with the same gene defect for biochemical investigations in other NIDR projects in other Institutes.

Methods Employed:

This investigation requires three approaches:

1. Analysis of census and other civil records.

2. Survey of groups by letters to physicians and public health officials concerned.

3. Field work by NIDR teams.

a. Analysis of census and other records:

Because of the U. S. Census Bureau's wish to further classify people whose race entries were somewhat nebulous, the 1950 Enumerators Reference Manual directed, "Report persons of mixed white, negro, and Indian ancestry living in certain communities in Eastern United States in terms of the name by which they are locally known". Such persons were then to be classified for publication purposes among "other nonwhite races", that is, other than Negro, American Indian, Chinese, Japanese, or Filipino. Starting with these data, and tracing the surnames so recorded through previous U.S. and British Colonial Census records, it was determined that such surnames frequently residing in the same household or neighborhood, showed variable race entries from decade

to decade, even for the same individual (i.e., white, Negro, Indian or other), and for the most part had large families. The existence of suspected tri-racial isolates from these data were then verified from local civil, historical, and social records such as marriage records, historical accounts, and newspaper articles. A search of the anthropological and social literature frequently revealed studies on such groups. Visits to these communities, to verify the existence and surname data, were made by collaborators and this investigator.

Of the 116 counties checked, the population of tri-racial character was estimated at 77,000 persons, of which 33,000 were listed as Indian, 29,000 as white, 14,000 as Negro and 1,000 under colloquial race names. Forty percent of the total reside in North Carolina.

Local and census records were used to estimate consanguinity and fertility. For example, census data from one North Carolina group showed the highest fertility ratio for any known racial or ethnic group in the United States. The ratio of children under 5 years old per 1,000 women, 15 to 49 years old, was 825 as compared to 417, the U.S. average. Marriage records of one group showed that 17% required ecclesiastical dispensation for relationship within the first three degrees (ie., 2nd cousins or closer). Investigation of the marriage records in Barbour and Taylor Counties, West Virginia, showed that 102 of the 112 marriages from 1856 to 1931 were to other tri-racial group members. School records of the same group in 1958 showed an unusual proportion of students from the group in the higher age levels for the grade in which they were in.

b. Survey of groups by letters to physicians and public health officials:

After determining the number and surnames of persons comprising these isolate populations, letters were sent to all county health officials in the 116 counties where these people reside requesting information on specific listed hereditary illnesses among these groups and any illness that appeared unusually frequent in such populations. Other local physicians and dentists were also contacted. These returns were tabulated. Literature by all writers was scanned for mentioned hereditary illness in these groups, (e.g., microphthalmia in the Moors of Delaware).

c. Field work by NIDR team:

It is planned to send investigators to these areas for 1-3 day preliminary reconnaissance work. If any important leads develop or if the reconnaissance shows promise of further study, these studies will be planned around the genetic material found. Some of this work has been done. For example, a visit to Halifax County, North Carolina, revealed a very rare hereditary eye lesion that has not, to my knowledge, been reported. A detailed clinical medical genetic study has been completed for the eye disease in this group.

Patient Material:

Patients found in this study of isolates have been admitted, from time to time, for the study of particular diseases.

Major Findings:

1. The reported high incidence of harelip and cleft palate in the Guinea group of West Virginia was not verified by our investigating team.

2. A hereditary form of benign intraepithelial dyskeratosis of mucosa and conjunctiva was found to affect 76 members of the Haliwar group in North Carolina. An unusual and undiagnosed hereditary neurological disease was also present in these people.

3. The reproductive rate of the Lumbee Indians of North Carolina is the highest of any ethnic group in the United States.

4. The size, location, distribution, emigration pattern, and a knowledge of the hereditary diseases present in many of these groups has been further described.

5. Several studies have been initiated on these groups by other investigators to whom we furnished the basic population data, as to location, surname, and population size. These studies have already shown several unusual hereditary conditions present in these groups, including hemoglobin disorders and sexual intermediates.

Significance to Dental Research:

These groups offer unusual genetic material for the study of oral connective tissue, eye, and metabolic defects in sufficient quantities so that the geneticist can assure the clinical and biochemical investigator that the genes involved are all of the same type. This offers an epidemiological method for the selection of patients with hereditary disease all with the same metabolic defect.

Proposed Course of Project:

To continue to accumulate information on the hereditary diseases present in these isolate populations.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Beale, C.L.: American Tri-racial Isolates; Their Status and Pertinence to Genetic Research. Eugenics Quarterly 4:187-196, December 1957 (Published after 1957 project report)

Honors and Awards relating to this project: None

Serial No. NIDR-48 (c)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Hereditary Defects of Enamel and Dentin
(Michigan Study)

Principal Investigator: Dr. C.J. Witkop, Jr.

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1/4
Professional: 1/4
Other: None

Project Description:

Objectives:

1. To define the various hereditary defects in enamel and dentin.

2. To determine the prevalence and mutation rates in the general population.

3. To determine the mode of inheritance.

4. To investigate the histological processes involved in the defects.

5. To describe any associated physical or chemical defect.

6. To investigate possible linkage associations.

7. To determine the various methods of restoration, prevention or treatment where possible.

Methods Employed:

1. Field Study: A survey of 96,761 school children, between the ages of 4 and 12, was conducted in 42 counties of the State of Michigan for hereditary

Part A (continued)

defects and other formations. Subsequent pedigree studies on these individuals were conducted to determine the mode of inheritance and possible linkage with known genetic markers, such as blood type, secretor factor, and P.T.C. taste test. These defects were classified by clinical descriptions, histological findings, and inheritance patterns. Patients with representative difficulties were brought to NIH for clinical evaluation.

2. Clinical Study: Complete physical and routine laboratory examinations were given to determine if any physical or metabolic defect was also associated with the tooth defect. Except in one particular defect, osteogenesis imperfecta, no genetic defect was found associated with the tooth condition.

3. Laboratory Study: Histochemical studies conducted on fresh ground and decalcified specimens using normal controls, showed that there was a possible biochemical defect in opalescent dentin. Linkage studies were analyzed.

Patient Material:

Patient material was obtained from 1) individuals contacted during our field study in Michigan; 2) individuals in our Genetic Study in Southern Maryland, Project No. D-4-002; and 3) in a few instances from individuals referred by local practitioners.

Major Findings:

1. The mutation rate for opalescent dentin is extremely low. No case that could be a new mutation was found among the 42 propositi in this study. A check of other cases reported in the literature and by other investigators failed to find a single instance of this disease that could possibly have arisen by mutation. This is the only autosomal dominant hereditary condition in man in which a mutant has never been found.

2. The homozygous state of opalescent dentin may be lethal causing intrauterine deaths.

3. From a comparison of the Michigan population with the Maryland population, one of two possibilities

Part A (continued)

exists. Either the defect known as opalescent dentin is the same in both populations, with the gene manifesting more severe symptoms because it is present in a different gene complex in the Maryland population; or the gene is different in the Maryland population and, therefore, a different disease. If the genes are the same, the significance would indicate that defective genes adapt over many generations to the rest of the gene complex, and when put into a stock with different racial background, manifest themselves in a more severe form.

4. Some evidence is present that a bone defect of a mild type is found in people with opalescent dentin.

5. Found during this survey was a hereditary, tumor-like condition of the teeth closely associated with dens-in-dente, which is also probably hereditary, and with odontomas, which have been described as dentin dysplasia. This is inherited as an autosomal dominant trait.

6. Dens-in-dente shows a familial pattern probably inherited as a dominant trait.

7. We have found a hereditary condition consisting of premature closures of the fontanels, ptosis of the eyelid, absence of tear ducts, and missing lateral incisors that is inherited as an autosomal condition. These individuals require prompt surgical intervention for the cranial defect. Many also need surgical correction of an associated agenesis of the condyles of the mandible.

Significance to Dental Research:

The etiology of several conditions may now be ascribed to hereditary causes. The clinical description of the oral defects has been given, and experience in surgical correction and restoration has been obtained.

Proposed Course of Project:

We recently have initiated, with Dr. Karl Piez, NIDR, an investigation to try to determine the biochemical defect present in opalescent dentin. Histochemical studies indicate that there are more chemically reactive groups in the matrix of the dentin in the

Part A (continued)

affected teeth than in normal teeth. A basophilic P.A.S. positive substance is present in dentinogenesis imperfect similar to that found in the bone of osteo-genesis imperfecta. We believe that this is an abnormal protein. The chemical methods are being perfected for analysis of normal bone, dentin, skin, and tendon, which will be utilized to investigate a variety of hereditary defects. The first of these will be opalescent dentin. Continued accumulation of pedigree material and linkage data is planned.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Witkop, C.J., Jr.: Genetics and Dentistry: Eugenics Quarterly, 5:15-21, March, 1959.

2. Witkop, C.J., Jr.: Genetics and Dentistry (In Hammons, Helen, G.ed. Heredity Counseling Hoeber, N.Y. 1959) pp. 13-28.

Honors and Awards relating to this project: None

Serial No. NIDR-49 (c)
1. Clinical Investigation
2. Human Genetics
3. Brandywine, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Genetic Study in Southern Maryland.

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: Dr. Laurence Schneiderman
Dr. Robert O. Wolf
H. R. Dyson, R.N.
Dr. A. Donald Merritt

Cooperating Units: Blood Bank DBS; NIAID; NCI; School of Dentistry, Howard University; Sociology Department, Catholic University.

Man years (calendar year 1959):
Total: 3½
Professional: 1½
Other: 2

Project Description:

Objectives:

1. To determine the total amount of hereditary illness present in an isolate population of not over 5,000 related individuals of white, American Indian, and Negro ancestry as revealed by dental and physical examinations and selected laboratory procedures.

2. To determine the prevalence, mode of inheritance, effects on viability and longevity, clinical manifestations and variations in expressivity of the hereditary illnesses found.

3. To investigate the relationships and possible linkages between these illnesses and blood types, secretor factor, P.T.C. taste test and other genetic markers.

4. To investigate genetic factors in illness not usually known to have a hereditary component.

Part A (continued)

5. To select patient and biological material from this population for clinical and biochemical investigations of specific illnesses and normal factors (these will constitute separate projects).

6. To assess the effects of inbreeding as revealed by in-group and out-group marriages and by degree of relationship (i.e., first cousins, first cousins once removed, second cousins, etc.) as shown by perinatal deaths, viability, longevity, and the presence of apparent homozygous recessive illness.

7. To try to trace the source of outflow of pathological genes in this population.

8. To assess the social and genetic factors in mate selection.

9. To look for correlations between genetic illnesses and other normal and abnormal factors that may have etiological or diagnostic relationships.

10. To try to reconstruct, on the basis of gene frequencies, the sources of the group's genetic material.

11. To compare the prevalence of illness, gene frequencies, and vital statistics with other isolate populations and the general population.

12. To detect carriers of recessive genes, if possible.

Methods Employed:

This study is divided into three phases:

1. Field study:

The field study consists of history and dental, medical and laboratory examinations designed to detect as much hereditary illness as possible.

a. History, genetic and census data are obtained on a questionnaire covering 158 items which arranges the data by family groups and by sibships. Such items as birth order effects, parental age, consanguinity, age of onset, longevity, and perinatal mortality are included. A record of hospitalizations and institutionalizations is obtained in addition to a medical and genetic history. A master kindred chart showing the relationship of each individual is made.

[illegible] (continued)

b. A search of existing social and church records for births and marriages is made by Fr. Thomas Harte. Sociological data on development in marriage patterns in this group are also obtained.

c. All subjects in this study are then given a complete dental and medical examination, designed to detect as much hereditary illness as possible. This includes complete blood typing genetic markers; urine analysis; hematological work-up, especially for abnormal hemoglobins; and special procedures such as X-rays and ophthalmological examinations where indicated. These examinations are made during the summer months at mobile units.

d. All data are then coded and tabulated for I.B.M. punch cards.

2. Clinical Study:

Selected patients seen in the field study are brought to NIH for detailed clinical studies. These studies are designed to give a description of the hereditary diseases involved; to determine the limits of the signs and symptoms of the illness; and to describe any metabolic abnormalities detectable on routine laboratory examinations, or other associated defects that might be found on X-rays or by special procedures.

3. Biochemical Investigation:

Selected genetic defects are being investigated biochemically to find the chemical error present. Currently under study are sickle cell disease, dentinogenesis imperfecta, and diffuse goiter. The sickle cell disease is being investigated by tonometry, electrophoresis, and the survival of this abnormal hemoglobin in transfused patients. Dentinogenesis imperfecta is being investigated histochemically and by chemical analysis for abnormal protein constituents. The goiter patients are being studied for the presence or absence of non-thyroglobulin thyroid proteins, the response to thyroid stimulating hormone, serum electrophoresis, total protein bound and butanol extractable iodide, and incubation of thyroid biopsies with I^{131}.

Part A (Continued)

Patient Material:

Patient material is selected from the families seen in the field studies. Approximately 500 hospital days have been utilized by this and other institutes.

Major Findings:

These data have not been analyzed statistically; however, a preliminary examination indicated the following:

1. There appears to be a hereditary block in thyroglobulin synthesis that accounts for the prevalence of goiter in about 8 percent of the women in this group.

2. A new hereditary bone disease, best described as generalized hereditary osseous hyperplasia, has been found in this group to be inherited as a recessive trait, and is known to exist in three other families. This is a newly described bone condition in which all deaths, with one exception, of people showing this disease have been attributed to respiratory causes.

3. Ankyloglossia appears to be inherited as a dominant trait.

4. There appears to be a genetic basis for certain speech defects not necessarily associated with gross oral abnormalities.

5. Four patients with a rare blood type, which has a frequency of .00008 in the general population, have been found.

6. Genetic evidence has been obtained to show that either there is an alternate pathway in melanin synthesis (other than that presently proposed) or that a secondary pigment system is present in humans.

7. A familial form of gynecomastia exists that is not Kleinfelter's syndrome.

8. The frequencies of dentinogenesis imperfecta and albinism in the Brandywine group are the highest known in any population.

Part A (continued)

Significance to Dental Research:

An isolate population has been found containing a vast number of hereditary diseases which may shed light on genetics and biochemical pathways of various hereditary defects.

Proposed Course of Project:

To continue the field, clinical and biochemical studies for the next years.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Dyson, Hazel R., Witkop, C. J., Jr., and Butters, Shirly S.: Genetics in Public Health Nursing. Eugenics Quarterly 5: 23-28, March 1958.

2. Carter, J. F.: A Description of some speech anomalies in a tri-racial isolate group. A Thesis, presented in partial fulfillment of the requirements for a Master of Science Degree, May 1958, University of Maryland.

3. Harte, T. J.: The Use of Parish Records in Social Research. Am. Catholic Sociological Review, June 1958.

4. Harte, T. J.: Trends in Mate Selection in a Tri-racial Isolate. Social Forces, November, 1958.

5. Dyson, H. R., Witkop, C.J., Jr., and Butters, S.S.: Genetics in Public Health Nursing. Chap. 3 (In Hammon, H., ed. Hereditary Counseling Hoeber, N. Y., 1959) pp. 29-41..

6. Witkop, C.J.,Jr.: Tyrosinase and Albinism, Am. J. Human Genetics, 11:91, March 1959.

7. Hursey, R. J., Witkop, C. J.,Jr., Miklashek, D., and Sackett, L. M. Dentinogenesis Imperfecta in a Racial Isolate with Multiple Hereditary Defects, Oral Surg., Oral Med., Oral Path. 9: 641-658, June 1956.

Honors and Awards relating to this project: None

Serial No. NIDR-50 (c)
1. Clinical Investigations
2. Human Genetics
3. Bethesda & Clinton, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Hereditary Eye Defects

Principal Investigator: Dr. C. J. Witkop, Jr.

Other Investigators: H. R. Dyson, R.N.

Cooperating Units: Laboratory of Ophthalmology, NINDB;
University of North Carolina; and
Ophthalmology Dept., Washington
Medical Center.

Man Years (calendar year 1959):
Total: ½
Professional: ¼
Other: ¼

Project Description:

Objectives:

To study the hereditary factors involved in eye diseases encountered in the Genetic Study in Southern Maryland and in the study of Tri-racial Isolates in Eastern United States. Three conditions have been encountered which have been included under this project:

1. A study of chronic simple glaucoma in the Southern Maryland population.

2. A study of hereditary benign intraepithelial dyskeratosis in the Haliwar population.

3. A study of cranial dysotosis and ptosis occurring in a Southern Maryland family.

These families were discovered during a study of hereditary diseases in isolate populations.

Methods Employed:

Field workers obtain history and kindred charting from the families concerned, make appointments for eye examinations, and record the data. All patients in the kindred are examined by the usual ophthalmological methods. Blood and saliva examinations, and PTC taste test are given for evaluation of family relationships and for linkage purposes.

Part A (Continued)

Patient Material:

Patient material is drawn from these families and admitted to the Ophthalmological Service of NINDB.

Major Findings:

1. Mrs. Hazel R. Dyson, R.N., assigned to the NINDR Human Genetics Section, has successfully demonstrated a genetic method for the prediction and detection of chronic simple glaucoma in individuals. She found that if an individual has an inherited disease, examination of predetermined members of the kindred may reveal additional examples of the disease and thus increase the effectiveness of screening procedures. Further, if the mode of inheritance is dominant, as with glaucoma, one can accurately trace the affected individuals through family history and home visits to identify the sibships that should be examined.

   For example, during the early stages of a current dental examination program of an isolate population group in Maryland, two patients were found to have chronic glaucoma. A family history of eye disease was obtained for each, and sibships in which glaucoma was most likely to occur were established. Examination of the selected kindred revealed five cases of frank glaucoma and one glaucoma suspect. Two of the patients were discovered before they were aware of any eye trouble. Additional studies on other families have shown that relatives of affected patients frequently have the disease.

   The Washington Society for the Prevention of Blindness is now utilizing the described method of study for detecting new cases of glaucoma.

2. A new hereditary disease has been investigated in detail and is in the process of being published. This disease, hereditary benign intraepithelial dyskeratosis of mucosa and conjunctiva, was found to occur in 75 individuals among the 514 descendants of a woman born in 1834 and who are today members of the Haliwar isolate of Halifax and Warren Cos., N. C. This disease has also been traced throughout the white collateral descendants of the family. This disease affects the oral mucosa and conjunctiva leading to blindness. The defect is one of cellular maturation. A diagnostic test for affected individuals was developed giving no false positive or false negative results. The genetics of the disease were analyzed in detail. The condition is inherited as an autosomal dominant trait.

Part A. (Continued)

Significance to Dental Research:

1. Genetic principles applied to such illnesses as chronic simple glaucoma provide a practical method for detecting this disease in an early stage when it is most susceptible to treatment.
2. Hereditary defects may affect several structures, and a clue to their diagnosis may be provided by dental practitioners.
3. A new hereditary disease affecting the eye and buccal mucosa was described in detail.

Proposed Course of Project:

Discontinued. Publication now in press.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Witkop, C. J., Jr., Shankle, C. H., Graham, J. B., Murray, M. R., Rucknagel, D. L. and Byerly, B. H.: Hereditary Benign Intraepithelial Dyskeratosis of Mucosa and Conjunctivae, Am. J. Path. (In press.)

Honors and awards relating to this project: None

Serial No. NIDR-51 (c)
1. Clinical Investigations
2. Human Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Sickle Cell Anemias and Other Inherited Hematological Disorders.

Principal Investigator: Dr. D. L. Rucknagel

Other Investigators: Dr. C. J. Witkop, Jr.

Cooperating Units: NIAMD

Man Years (calendar year 1959):

Total:	1½
Professional:	½
Other:	3/4

Project Description:

Objectives:

To study in detail physiologic, biochemical, and genetic factors in various inherited hematologic disorders in the Brandywine tri-racial isolate. Laboratory and clinical data will be integrated with the pedigree and analyzed for the purpose of defining biochemical mechanisms and mode of inheritance of disease.

Methods Employed:

Field surveys among the Wesort groups have detected individuals with several hematologic disorders. Approximately fifteen percent of this group harbor the sickle cell gene. Individuals with sickle cell anemia are hospitalized and evaluated by standard hematologic procedures. In addition, specialized investigations of intravascular sickling, arterial oxyhemoglobin saturation, and erythrocyte physiology using tonometric techniques are utilized.

Relatives of propositi are studied hematologically using standard hematologic techniques, paper and starch block electrophoresis and, when indicated, oxygenation kinetic studies.

Part A (continued)

Non-sicklemic anemic individuals and their families are also being studied to elucidate other genetic abnormalities. One individual with pernicious anemia was studied extensively (in cooperation with Drs. Seegmiller, LaDu and Laster) in an attempt to elucidate the effect of vitamin B_{12} deficiency upon purine biosynthesis.

Patient Material:

This year another 1100 individuals were screened in the field examinations. Anemic individuals were selected from this group for the most part. A few non-group sickle cell anemia patients were studied as control subjects. Fetal hemoglobin assays on blood of patients with choriocarcinoma are continuing, and additional studies are also under way on patients with an assortment of other malignancies being treated by the National Cancer Institute.

Major Findings:

1. During the past year serial clinical studies and erythrocyte oxygenation investigations have been performed on eighteen individuals with sickle cell anemia. These have indicated that the amount of intravascular sickling and the degree of arterial oxygen unsaturation are reasonably constant in a given individual over a long period of time. Quantitative studies have documented several degrees of severity of these disease manifestations. Moreover, laboratory investigations have demonstrated that the variations in clinical manifestations and in arterial saturation can be correlated with abnormalities in erythrocyte physiology per se, and not with associated systemic disease, ie., pulmonary insufficiency, cardiac anomalies, etc.

2. The patient with pernicious anemia was found to excrete large amounts of formimino glutamic acid, a compound involved in folic acid metabolism, since it is also excreted by rats and humans receiving the folic acid antimetabolite, amethopterin. In addition, carbon-14-labelled formamidino aminoimidazole carboxamide was incorporated less efficiently into uric acid than aminoimidazole carboxamide in our patient. Both of these observations implicate defective formyl group metabolism in pernicious anemia.

Part A (continued)

3. To date, increased amounts of fetal hemoglobin have been found in 10 of 35 patients with choriocarcinoma studied. Bloods from 30 individuals with other malignancies contained normal amounts of fetal hemoglobin. Since chorionic gonadotropin is considered the stimulus to fetal hemoglobin production in these patients, it may also increase the fetal hemoglobin content of cells of patients with sickle cell anemia, thereby alleviating the clinical severity. However, preliminary trials on a small number of sickle cell anemia patients have shown no sifnificant results to date.

Significance to Dental Research:

Since it is recognized that genetic factors are important in the study of dental diseases as well as other medical problems, this program is aimed at evaluating genetic mechanisms by as many approaches as are feasible. By studying specific entities as thoroughly as possible and correlating variations of disease and laboratory findings with pedigree data, it is anticipated that conclusions may be forthcoming regarding genetic mechanisms.

Proposed Course of Projects:

Enlargement of the pedigree will continue but hematologic investigations are now being concentrated in areas of the pedigree deemed to be most promising. These are designed to elucidate the presence and nature of modifying hereditary factors in sickle cell anemia, and to evaluate the presence of Thalassemic genes in the non-sicklemic anemia sibships. Data now being processed and written up.

Other specific projects planned include:

1. Extension of sickle cell erythrocyte physiology investigation.
2. Formyl metabolism in pernicious anemia.
3. Erythrocyte enzyme anomalies.
4. Haptoglobin genetics in the Brandywine Group.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project.

Rucknagel, D.L., and Witkop, C.J., Jr.: Correlation of clinical status with arterial oxyhemoglobin unsaturation in patients with sickle cell anemia. Blood. (In press).

Honors and awards relating to this project: None

Serial No. NIDR-52 (c)
1. Clinical Investigation
2. Human Genetics
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Oral Aspects of the Results of Consanguineous Marriage in Hiroshima and Nagasaki, Japan.

Principal Investigator: Dr. J.D. Niswander

Other Investigators: Dr. C.J. Witkop, Jr.

Cooperating Units: National Academy of Sciences, Washington, D.C., under the direction of James V. Neel, William J. Schull, and James N. Spuhler.

Man Years (calendar year 1959):
Total: 1
Professional: 1
Other: None

Project Description:

Objectives:

1. In connection with the genetic program of the Atomic Bomb Casualty Commission in Hiroshima and Nagasaki, Japan, the details of which have been presented by Neel and Schull (Neel, J.V. and Schull, W.J.: The Effect of Exposure to the Atomic Bombs on Pregnancy Termination in Hiroshima and Nagasaki. National Academy of Sciences, #461, Washington, 1956), the outcome of pregnancy was determined in 5,033 instances in which the parents reported consanguinity of some degree. The products of these pregnancies would, if now alive, range in age between five and ten years at this time. In essence, this proposal involves an attempt to obtain a detailed follow-up study on each of these 5,033 pregnancy terminations, plus a suitable control material.

2. Of the 5,033 pregnancy terminations, a number are known to have resulted in stillbirths or deaths during the first nine months of life. Starting from the records of the Atomic Bomb Casualty Commission, a trained staff of "field workers" will attempt to re-establish contacts with all of the remaining children (i.e., registered in the Genetics Program and not known to have died during the period of time covered by this program). This attempt will result in a roster of living children, a roster of now-deceased children, and a roster of children who have moved from the two study cities. An effort

will be made to establish cause of death for all deceased children. The background of the children who have moved from the cities will be analyzed, in a search for biasing factors. It is possible that an attempt will be made to contact these latter children with reference to the question of present viability. The fact of death and, possibly, the cause of death will be analyzed in relation to parental consanguinity.

3. A pilot study carried out in 1956 under the direction of W.J. Schull has already revealed that the death rate among the children of consanguineous marriages is approximately twice the control death rate (cf. Appendix). There is, then, already considerable assurance of significant results from this inquiry. These same data can be analyzed in relation to the radiation history of the parents, thus yielding additional information concerning the genetic effects of the atomic bombs.

4. The dental portion of this study proposes to determine the effects of consanguinity on DMF rates, periodontal disease indices, growth and development problems, and specific hereditary diseases affecting the oral cavity.

Methods Employed:

Using ABCC facilities in Nagasaki and Hiroshima, a team consisting of specialists in such fields as pediatrics, hematology, dentistry, and anthropology began examinations of offspring of consanguineous marriages and appropriate control groups in September, 1958. The following examinations are performed:

1. Physical examination: This consists of a standard physical examination, with emphasis on characteristics which can be graded in an objective manner lending itself to statistical analysis.

   a. Presence of gross physical defect; i.e., abnormalities of skin, musculature, skeleton, etc.

   b. Defects of the auditory mechanism, with special reference to loss of hearing.

   c. Defects of the eye, including loss of visual acuity.

   d. Cardiac abnormality (cardiomegaly, disorders of rhythm, murmurs, etc.).

   e. Pulmonary abnormality.

   f. Apparent abnormality of abdominal viscera.

   g. Abnormality of external genitalia.

h. Neurological evaluation.

i. Bone age (single wrist film).

j. Determination of leucocyte count and hemoglobin level.

k. Standard urinalysis.

2. The dental examination includes pathology of the soft tissues, pathology of the teeth and index of malocclusion, index of tooth loss, developmental failures of oral structures, DMF and DEF rates, and the association of these conditions with and without generalized hereditary disease.

3. Anthropometric examination: This will include such standard items as weight, sitting and standing height, span, measurements of head and face, subcutaneous fat index, etc.

4. Psychometric examination: Discussion here centers on the use of the Koga intelligence test, one of the Japanese equivalents of the Stanford-Binet. If possible, all tests will be individually, or semi-individually, administered.

All results will be coded and transferred to IBM cards for statistical analysis. As for the Genetics Program data, the statistical analysis will undoubtedly draw heavily on an approach which makes allowance for certain uncontrollable sources of bias. The first result will be a comparison of the different groups of children with respect to a wide variety of characteristics with particular reference to the regression of the findings of degree on inbreeding. Beyond this, however, it is anticipated that the material will permit tentative conclusions concerning the number of "lethal-equivalents" carried by the average Japanese, utilizing the mathematical approach suggested by Morton, Crow, and Miller (1956).

Major Findings:

About half of the proposed 5,000 offspring of consanguineous marriages and the control group have been examined. There is no startling difference in the prevalence of known hereditary defects between the two groups. From normative data on Japanese children of Hiroshima, it has been determined that between 1950 and 1958, for 10 year olds, there has been an average of 3.35 cm. increase in stature. Between 1951 and 1959 there were significant increases in stature, weight and number of permanent teeth erupted for 6,8, and 10 year old children.

Significance to Dental Research:

1. Properly executed studies on consanguinity effects will yield information on the genetic structure of human populations which at present can be obtained in no other way. Such information is vital to a realistic, quantitative appraisal of such problems as are posed by the increasing exposure of the human species to ionizing radiation.

2. The Japanese Study will offer an opportunity to study the genetic effects of a specific type of inbreeding, complementing our Brandywine Study. Whereas, the latter program is assessing the effects of continuous inbreeding the Japanese Study has the unique advantage of affording an evaluation of one type of mating pattern.

3. It would be expected that certain recessive characteristics determining normal growth and development, as well as pathological traits, would show up in the homozygous state with increased frequency. Many of these conditions are probably unknown, and the Japanese Study consequently offers an opportunity to define both normal factors and pathological traits.

4. The Brandywine population group is descended from relatively few ancestors. Consequently, unless an ancestor carried a hidden trait it could not appear in the descendants, that is, barring mutation. Therefore, the Brandywine group would present only those inherited conditions carried by predecessors. On the other hand, the first cousins in Japan are drawn from a large unrelated population and, therefore, many characteristics and pathological traits should appear in their offspring.

Proposed Course of Project:

Examination will continue until July 1960, following which Dr. Niswander will return and spend the following year analyzing the data.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Niswander, J.D., and Sujaku, C.: Dental Eruption, Stature, and Weight of Hiroshima Children. J. Dent. Res., (In press)

Serial No. NIDR-53 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies of the Oral Microbiota and Oral Environment in Relation to Dental and Systemic Diseases.

Principal Investigator: Dr. R.M. Stephan

Other Investigators: None

Cooperating Units: Dental Clinic, Clinical Center, NIH

Man Years (calendar year 1959)

Total:	1¼
Professional:	¼
Other:	1

Project Description:

Objectives:

1. To study macroscopically and microscopically the occurrence growth, localization, morphology, physical characteristics, and color reactions of microbic plaques on the teeth, in gingival crevices, in periodontal pockets, and on the oral mucous membranes.

2. To determine differences in microbic plaque formation, food retention, salivary flow, gingival exudates, and pH levels which are associated with caries, erosion, and periodontal disease in comparison with normal dental conditions.

3. To investigate possible associations of specific types of oral microorganisms with dental and systemic diseases.

Methods Employed:

Direct stereomicroscopic observations in the oral cavity are made at 6X-40X magnifications with the Zeiss "Otoscope." Direct pH measurements are made with micro antimony and glass electrodes and with microapplicators containing colorimetric indicators.

Measurements of parotid salivary flow rates are made with the "Lashley Cup" apparatus. Samples of plaques and other surface material from the teeth, periodontal pockets, and mucous membranes are taken with dental explorers, scalers, swabs, and specially designed microscalers for quantitative samples which can be weighed in a microbalance. These samples are studied in micro culture slides by phase microscopy, in gram stained smears, and in selective culture media. These samples have also been used in the study of filamentous organisms by Dr. Arden Howell.

Patient Material:

One hundred and twenty-two patients have been studied this year. Most of the cases have been outpatients of NIDR referred for study of rampant caries, dental erosion, severe periodontal disease, or stomatitis. Patients with various diseases who have been referred to the Dental Clinic from the other Institutes at NIH also have been studied for possible interrelationships between oral conditions and other diseases, particularly those with xerostomia or Sjogrens Syndrome.

Major Findings:

1. Further information has been gained on the complex host-parasite relationships in the oral cavity, particularly as they relate to caries, erosion, periodontal disease and xerostomia. Quantitative estimates of the different forms of organisms in surface material removed from representative localized areas of the oral environment have demonstrated wide variations in the microbiota between different individuals, and between different areas in the same individual. At the same time, a more or less basic underlying pattern has been found for the distribution of certain common types of organisms, such as spirochetes in gingival crevices and streptococci on tooth surfaces. The most interesting findings have been the unusually heavy growth of microbic plaques in rampant caries and the presence of many different forms of motile organisms in severe periodontal disease. Some of these motile forms have not yet been grown in pure culture and they have not been identified in histological material, so that their importance and general distrubution

Part A (continued)

is not yet known. The growth of Endamoeba gingivalis and Trichomonas tenax in periodontal pockets has been observed by phase microscopy and by culture in egg media.

2. Studies on intra-oral pH have continued to show normal pH levels between 6.0 and 8.0. In active caries, pH levels have been as low as 4.2. On the other hand, pH levels of 5.4 have been found in an unusual type of nonadherent "plaque" in active dental erosion areas.

3. Patients who have received therapeutic X-ray irradiation of the head and neck have been of particular interest since, in some cases, they develop a lack of oral secretions and unusually rampant dental caries as a result of changes induced by the radiation.

Significance to Dental Research:

This project has been planned to cover a neglected area in oral pathology and microbiology--namely, to furnish information on the total microbiota in the oral cavity as it grows "in situ" and as it is related to clinical disease processes.

Proposed Course of Project:

1. This project has explored several methods for study of the oral microbiota and oral environment and it is planned to complete the analysis of the present data and to make follow up observations on salivary flow and rampant caries following X-ray therapy and the development of Sjogrens Syndrome.

2. Further study also is planned of the motile organisms in periodontal disease, and the pH changes in cases of erosion.

Part B included: No

Serial No. NIDR-54
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Interrelationships between Local and Systemic Factors in the Etiology of Dental Diseasesin Rats.

Principal Investigator: Dr. R.M. Stephan

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959):
Total: 1 1/4
Professional: 1/4
Other: 1

Project Description:

Objectives:

1. To determine the essential experimental conditions which control the development of dental caries and periodontal disease in rats.

2. To study the relation between caries in rat incisors, and bacterial plaque, tooth formation, food retention, and intra-oral pH.

3. To develop the use of vital stains and fluorescent dyes as indicators for the earliest stages of caries.

4. To explore the caries-inhibiting action of some antibacterial dyes and antibiotics which can be deposited in combination with calcified tooth substance.

Methods Employed:

1. Three basic diets have been used: a) a nutritionally defined diet of sucrose, casein, salts, and known vitamins which induces very extreme caries, b) a nutritionally defined diet which induces little caries, and c) a stock diet which does not induce caries.

Part A (continued)

2. In multifactorial experiments, these basic control diets have been supplemented with antibacterial and nutritional substances to determine systemic effects and the relation of growth rates to caries.

3. Glucose and sucrose have been added to the drinking water to produce both local oral and systemic effects.

4. Vital stains have been injected intraperitoneally, added to the diet, or added to the drinking water to serve as indicators for the early stages of caries. Stains have also been studied as indicators for pH and caries activity, and fluorescent effects of some stains and antibiotics have been observed in teeth using ultraviolet illumination under the stereomicroscope.

5. Bacterial plaque formation has been studied microscopically on the incisor teeth in the live animal, and direct pH measurements of the plaques have been made with micro-antimony and glass electrodes and with pH indicators.

Major Findings:

1. Further observations have been made on a number of vital stains which have been found to be good indicators for the initial stages of caries. One of the most interesting findings is that some dyes stain only the very earliest stages of caries in enamel whereas others stain only the advanced stages of caries. Although the hydroxyanthraquinone dyes continue to yield the best stain for bones and teeth, dyes from the oxazin and xanthene groups have been found useful for staining caries. There are several basic chemical reactions which can be demonstrated in the caries process by improved vital staining methods. Ultraviolet illumination has revealed fluorescent staining with substances such as aureomycin which combine with calcified tooth substance but have too little color to be visualized by ordinary illumination.

2. The effect of additions of sucrose and dextrose to the diet and drinking water has greatly increased caries in some experiments, and only moderately increased it in others. This has been shown to be

Part A (continued)

due partly to the age of the animal and to the fact that when sugar is given early, the effect on caries is greatest.

3. Attempts to increase caries in rats by inoculating with bacteria from the mouth of people with rampant caries have not succeeded.

4. These studies have continued to demonstrate that the development of caries in rats results from the interaction of many different factors including the strain of rat used, the physical properties of the diet, the nutritional value of the diet, the retention of diet in the oral cavity, the growth of microorganisms on the teeth, and the relative growth and activity of the individual animal.

Significance to Dental Research:

The development of improved methods for studying the etiology of dental caries and periodontal disease in laboratory animals will permit more precise testing of the relative importance of many factors which may potentiate or control these diseases. The use of vital stains will aid in the early detection of dental caries and serve as an indicator for tooth formation in relation to caries susceptibility.

Proposed Course of Project:

It is clear that both dental caries and periodontal disease are very complex problems and that additional new methods are needed for determining their activity. It is planned to continue multifactorial experiments in animals to further delineate the interrelationships of different types of factors in the development of dental diseases.

Part B included: No

Serial No. NIDR-55 (c)
2. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies on Enzymatic Reactions of Cyclic Alcohols and Steroids.

Principal Investigator: Dr. A. D. Merritt

Other Investigators: None

Cooperating Units: Dr. G. M. Tomkins, NIAMD
Dr. A. Karmen, NHI

Man Years (calendar year 1959):
Total: 3/4
Professional: 1/4
Other: 1/2

Project Description:

Objectives:

1. To further define the substrate specificity of liver alcohol dehydrogenase.

2. To specifically identify the stearic and conformational characteristics of substrates for liver alcohol dehydrogenase.

Methods Employed:

A rat liver enzyme was prepared by standard procedures (homogenization, fractional ammonium sulfate precipitation, dialysing), followed by chromatography on diethylaminoethyl cellulose. Assays of enzymic activity were made by chemical, gas chromatographic and paper chromatographic techniques, as well as following the ultraviolet absorbtion of diphosphopyridine nucleotides.

Major Findings:

Previous investigations by Dr. G. M. Tomkins indicated that cyclic secondary alcohols were able to oxidize reduced diphosphopyridine nucleotide in the presence of a rat liver homogenate. Studies were undertaken to purify the enzyme responsible for this activity. A partially purified enzyme was prepared and its kinetics, pH optimum, equilibrium constants, and substrate specificity were studied. Cyclohexanone was found to be stoichiometrically and reversibly reduced by diphosphopyridine nucleotide.

The products of the reaction were identified by means of paper chromatography. Since the substrate specificity paralleled that of horse liver alcohol dehydrogenase, the enzyme was thought to be a liver alcohol dehydrogenase.

While investigating the oxidation of cyclic secondary alcohols by a rat liver preparation, it was found that horse liver alcohol dehydrogenase had activity with these substrates. Cyclohexanol was a somewhat better substrate than ethanol on a molar basis. The kinetics of this reaction were examined.

Methyl substituted cyclic alcohols and ketones also were found to be active with liver alcohol dehydrogenase. The additional substituents allow the formation of cis- and trans- isomers which may be identified by means of gas chromatography.

Significance to Dental Research:

These studies have broad biological import and tend to broaden the basic biochemical and medical aspects of disease.

Proposed Course of Project:

Studies of cyclic ketone reduction products of liver alcohol dehydrogenase will be made to further define enzyme-substrate interactions.

In addition, some of the above techniques will be utilized in the study of ^{4} reduction on ring A of steroid hormones.

Previous studies by Dr. G. M. Tomkins on this enzyme indicated that there were a multiplicity of enzymes responsible for this activity. Attempts will continue in the separation of these enzymes into relatively pure components and then in the preparation of antibodies to these proteins in an effort to define similarities between the different enzymes. In addition, it is planned to study the antigenic relationships to a similar system of enzymes found in the microsomal fraction of rat liver homogenates. The two enzyme systems differ in that the soluble enzymes reduce Δ^4 bonds to 5β isomers while the microsomal enzymes form the 5α isomers. Amino acid analysis and the definition of the active center for these enzymes will be attempted.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Merritt, A. Donald and Gordon M. Tomkins: Reversible oxidation of cyclic secondary alcohols by liver alcohol dehydrogenase. The Journal of Biological Chemistry, 234:2778-2782, October, 1959.

[illegible]
1. Clinical Investigations
2. Medical Investigations
3 Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the Urinary Excretion Products of Histidine in Patients with Liver Disease.

Principal Investigator: Dr. A. D. Merritt

Other Investigators: None

Cooperating Units: Dr. Milton Silverman, NIAMD

Man Years (calendar year 1959):
Total: $\frac{1}{4}$
Professional: $\frac{1}{4}$
Other: 0

Project Description:

Objectives:

1. To test the sensitivity of histidine loading as an indication of liver dysfunction.
2. To determine the effects on this test of cyanocobalamine, folic acid, and methionine.

Methods Employed:

Patients with liver disease of variable etiology are fed a standard dose of histidine. Urine, prior to and following histidine, is analyzed by a microbiological assay for metabolic products of histidine. A complete history and physical examination, blood counts, urinalysis, and multiple liver function tests are obtained on all patients.

Major Findings:

Formimidoglutamic acid (FGA) and urocanic acid have been identified in patients with liver disease, and unusual amounts of these substances are found following oral loading with histidine. Normal patients do not exhibit these findings. The products (FGA and urocanic acid) have been isolated and identified by their melting points and ultraviolet and infra-red spectroscopy. The sensitivity of this functional capacity of the liver as an indication of liver disease is being investigated and compared with the usual liver function tests.

No effects have been found following the administration of cyanocobalamine, folic acid, and methionine.

Part A (continued)

Significance to Research:

The degree of liver dysfunction, particularly minor variations, may prove measurable with this test. The pathways of histidine metabolism in man will be partially defined.

Proposed Course of Project:

The gross findings will be defined further, and attempts to reverse the inability to utilize histidine completely will be made.

Part B included: No

Serial No. NIDR-57 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Serial Cephalometric Study of the Longitudinal Growth of the Soft Palate and Nasopharynx in Cleft Palate Children.

Principal Investigator: Dr. F. J. Coccaro

Other Investigators: None

Cooperating Units: Dr. Samuel Pruzansky, Assoc. Director, University of Illinois, Chicago Professional Colleges, Cleft Palate Center.
Dr. J. D. Subtelny, Eastman Dental Disp., Rochester, New York.
Mr. Carl White, Statistician, Biometrics Division, NIDR.

Man years (calendar year 1959):
Total: 3/4
Professional: ½
Other ¼

Project Description:

Objectives:

1. To study incremental changes in velar length from birth through 7 years of age in cleft palate subjects.

2. To analyze the growth pattern of the soft palate during the first 7 years of development for cleft palate individuals.

3. To make a comparative analysis between normative data obtained in the growth pattern and incremental changes occurring in the soft palate with data observed in these areas for cleft palate individuals.

4. To determine incremental changes in the horizontal and vertical dimensions of the nasopharynx that are incidental to growth during the first 7 years of life for cleft palate children.

5. To discern whether or not growth of the velum and the nasopharynx in cleft palate individuals differs significantly from that observed in the normal.

6. To determine the effect of palatal surgery upon velar length and the growth pattern of the soft palate.

Methods Employed:

1. The method employed for this study was based on serial cephalometric roentgenology. The technique used for taking the lateral headplates is well known and has been described by Broadbent, who helped develop the Broadbent-Bolton Cephalometer. A modification of the original cephalometer was designed to facilitate positioning the infant's head into the specially constructed headholder which is in a fixed relation to the X-ray tube. The newly designed instrument was built around a table instead of a dental chair, and only one tube (the 37 lateral) was used.

2. Since the last project report, an additional 20 cases have been included in the present study to increase the total number to 57.

3. Some 600 cephalometric lateral roentgenograms were obtained from the files of the Cleft Palate Center of the University of Illinois. These films were traced and analyzed. Classification of clefts was according to specific anatomical areas affected and grouped according to the type of cleft lip and/or palate involvement.

   Type I - Posterior clefts. These included clefts of the soft palate only and/or clefts of the soft palate and the vault of the hard palate as far forward as the anterior palatine foramen but not to include the alveolar process or the lip. Fifteen cases were included in this group, and a total of 150 lateral headplates were analyzed.

Part A (continued)

Type II - Unilateral clefts. In this group the lip and the palate are involved and either side of the face may be directly affected. In these cases the vomer is deviated from the midline at the line of attachment to the palatal process on the non-cleft side. Twenty-one cases were included in this group and a total of 210 cephalometric roentgenograms were traced and analyzed.

Type III- Bilateral clefts. These cases had clefts of the lip and palate on both sides of the midline of the face. The premaxillary segment is projected considerably forward of the profile and there is a complete separation of this segment from the lateral palatine processes of the maxilla as well as the horizontal processes of the palatine bone. There is no fusion of the palatine processes with the inferior border of the nasal septum. Ten cases were included in this cleft palate group and a total of 120 cephalometric lateral roentgenograms were analyzed.

Type IV - Alveolar clefts. These may be defined as clefts of the alveolar process, varying in degree of severity and usually associated with a cleft lip. Since there is no observable defect of the hard and/or soft palate, it permitted consideration of this sample as an additional control group. Eleven cases were included in this group and a total of 120 films were analyzed.

For normal controls, comparisons will be made with normative data on the growth of the soft palate reported by Subtelny. He studied 30 subjects with serial cephalometric X-ray records from infancy to early adulthood.

The cephalometric roentgenograms employed in this study were placed upon an illuminated tracing table and covered with transparent matted acetate paper and the anatomical landmarks used in the investigation were traced. Correctional scales cut from each film were utilized and the following linear measurements were determined.

A. Velar Length

The growth in length of the soft palate was evaluated by different measurements.

1. The linear distance from the posterior border of the hard palate, as visualized on the lateral headplate, to the tip of the uvula of the resting soft palate was measured. (PNS-U)

2. The linear distance from the point of intersection of the palatal plane with the pterygomaxillary fissure, to the tip of the uvula of the resting soft palate was measured (PTM-U).

Two reasons are projected for employing both the posterior border of the hard palate and the pterygomaxillary fissure in making velar length measurements. When the posterior border of the hard palate was used to define the anterior limits of the soft palate, it made it possible to determine the actual length of the soft palate in children with clefts of the palate. When the pterygomaxillary fissure was used to define the anterior limits of the soft palate, it became possible to measure the amount of soft palatal tissue existent within the defined skeletal framework of the nasopharynx.

B. Velar Thickness

To measure the thickness of the soft palate, a line was constructed perpendicular to the line representing the length of the velum (PNS-U). This line was drawn in the area of the greatest velar thickness.

C. Nasopharyngeal Measurements

Vertical Measurements: To study the increase in height of the nasopharynx as a result of growth, the linear distance from the point of intersection of the palatal plane with the pterygomaxillary fissure, to the cranial base plane (nasion-basion) was measured. This line was constructed perpendicular to the cant of the Frankfort Horizontal Plane and projected to intersect the cranial base line.

D. Horizontal Measurements

Growth in depth of the nasopharynx was evaluated by measuring the linear distance, along the palatal plane, from the point of intersection of the palatal plane with the pterygomaxillary fissure to the soft tissue of the posterior wall of the pharynx.

Major Findings:

1. The data indicates that all cleft palate groups demonstrated marked and consistent increases in velar length from birth up to approximately three years of age.

2. Periods of less active growth are noted from three to five years of age for all cleft palate groups.

3. Renewed growth activity is apparent, for all cleft palate groups, from five to seven years of age.

4. Although differences were recorded in velar length measurements between the cleft palate groups, these differences were determined to be not statistically significant at any of the age levels analyzed.

5. When compared to the normal, all cleft palate groups showed shorter velar length measurements from birth through seven years of age. These differences were determined to be statistically significant.

6. Rapid growth in velar length for cleft palate and normal groups is quite apparent during the first one and one-half years of life.

7. Differences in the velar growth pattern were noted when the cleft palate groups were compared to the normal control group. Periods of active and less active growth did not parallel each other when the cleft palate group was compared to the normal control group.

8. The essential parallelism existing between the growth curves for the cleft palate groups and the control groups before and after the mean age at which time palatal surgery was performed, reflects little, if any, significant effect of surgery upon velar length and the growth pattern of the soft palate for the periods studied in cleft palate children.

Part A (continued)

9. Vertical growth of the nasopharyngeal area was steady and consistent from birth through seven years of age with the greatest increment in vertical dimension noted from birth to 1½ and 2 years of age for the total cleft palate population.

10. Horizontal growth of the nasopharyngeal area was also studied and the data showed that increases in the anteroposterior dimensions of the nasopharynx were apparent, but not too well defined for the first year of life. From the first year to the seventh there were periods of apparent increase and decrease in the anteroposterior dimensions of the nasopharynx. Fluctuations in depth may be correlated with periods of adenoid growth and growth of the upper face.

11. Shorter vertical nasopharyngeal measurements for the cleft palate group at most of the age levels studied were noted when compared to the control groups.

12. Shorter horizontal nasopharyngeal measurements for most of the age levels studied were noted when the cleft palate group was compared to the normal control group.

Significance to Dental Research:

1. The cleft palate patient presents many complex oral-dental and facial deformities affecting mastication, speech, respiration and deglutition.

2. The need for basic information on growth and development of structures involved in these deformities is quite apparent.

3. Instituting therapeutic measures to produce adequate physiologic relationships of the anatomical parts in the velopharyngeal area is essential for the production of satisfactory phonetic articulation.

4. Adequate velar length along with the functional potential of velar tissue and its location to the posterior pharyngeal wall have been advanced as important factors for the production of satisfactory speech.

Part A (continued)

Proposed Course of Project:

1. Present study has been extended to include the analysis of an additional 200 cephalometric roentgenograms. Thus the findings from 57 cases (with the analysis of 600 lateral cephalometric roentgenograms) shall be strengthened statistically.

2. Future studies are anticipated from the analysis of this material relative to the growth of velar tissue before and after surgery.

Part B included: No

Serial No. NIDR-58 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: General Anesthesia (Dental) Baseline Data.

Principal Investigator: Dr. E.J. Driscoll

Other Investigators: Dr. Gilbert Christenson

Cooperating Units: Anesthesiology Department, Clinical Center.

Man Years (calendar year 1959):
Total: 1
Professional: 3/4
Other: 1/4

Project Description:

Objectives:

Dental general anesthesia is different from ordinary hospital anesthesias in several important respects: a) The patients are generally ambulatory and not usually sedated; b) The operations are performed in as light a plane as possible; and c) The operations are performed in or about the airway.

1. Because of these major differences, the background of knowledge in general dental anesthesia is not for the most part complete, accurate or comparable. Thus, a great need exists for a background of fundamental data concerning alterations in the physiological mechanisms. Although body functions may be altered within certain limits, great hazards may be encountered when these limits are exceeded. It is important, therefore, to define these limits.

2. The accumulated data from this study will also be used as a baseline of comparison for the many new anesthetic drugs which are being proposed for use in oral surgery.

Methods Employed:

1. For the purposes of standardization of surgical trauma, full mouth extractions have been utilized rather than random oral surgical cases. The teeth

Part A (continued)

are extracted in quadrants and the entire operation is standardized for procedure and traumatic experience.

2. During the current year, the new barbiturate Sodium Methohexitol has been evaluated, utilizing the same methods as in our Pentothal and Neraval investigations of 1958.

The parasympatholytic drug Trol (hexocyclium methyl sulfate) was employed for control of secretions in about half of the cases and atropine was used in the remainder. They were given intravenously three minutes before the barbiturate administration.

3. Supplemental information is still being obtained by combining the barbiturates with nitrous oxide-oxygen.

4. a. Physiologic status and alterations being studied and recorded are pulse, blood pressure, arterial O_2 saturation, respiratory phenomena, and cortical (brain) activity.

b. In addition, information is being gathered by continuous recordings of E.K.G., E.E.G., and an oximeter (which gives a continuous monitoring of arterial oxygen).

c. Important technical data, such as amount of drug used in units of time, length of operation and duration of sleep, are likewise recorded.

d. Pertinent patient data such as age, race, sex, and emotional status in relation to the conduct of anesthesia are being recorded, as well as post-anesthetic sequelae (headache, nausea and vomiting, depression and hiccoughs).

Patient Material:

This year we completed another 150 detailed studies on about 60 adult patients. We now have available for study and evaluation about 450 detailed records (E.E.G., E.K.G., pulse, etc.)

Major Findings:

Sufficient data is now available to determine objectively from brain wave activity the precise

Part A (continued)

anesthetic levels at which various procedures are being carried out with the three barbiturates Pentothal, Neraval, and Methohexitol.

1. Thus, it is definitely established that we are working in an extremely light plane. The E.E.G. is also making it possible for us to state with certainty the precise anesthetic plane which is present at specific intervals when other physiologic measurements such as pulse and pressure are made.

2. Both atropine and Tral reduced the number of arrythmias, as Banthine had done in our previous study.

a. Atropine was found to accelerate the pulse within a minute while Hexocyclium had no such accelerating property. However, when the barbiturate was given with both atropine and Tral, the acceleration took place.

Hexocyclium was found to be a better drying agent for oral secretions than atropine.

3. The blood pressure elevation and pulse acceleration noted with Pentothal and Neraval were greater when Methohexitol was used. The exact values are not yet available as the data has not been analyzed.

Significance to Dental Research:

1. The search is constantly being made in dentistry for safer general anesthetic agents. However, without baseline data on physiologic responses, this search has no foundation. Methohexitol is a good example of new drugs which are being introduced. (Methohexitol is due to be released in February 1960.) With our previous physiologic baselines on Pentothal and Neraval we are in a good position to evaluate Methohexitol.

2. The possibility also exists for improvement and refinement of present methods of anesthesia as well as laying a sound foundation for further anesthesiology research.

Part A (continued)

Proposed Course of Project:

1. The study will continue on good risk out-patients. Additional baseline and comparative data will be accumulated, studied and evaluated.

2. The study of Methohexitol will continue since it appears to have an excellent potential for use in Oral Surgery. The combination of nitrous oxide and Methohexitol will also be evaluated.

3. The effects of the vagolytic agents will be further recorded and observed.

4. Arterial Oxygen will be studied in more detail with the Oximeter.

Part B included: Yes

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Driscoll, E.J., and Christenson, G.R., and White, C.L. Physiological studies in General Anesthesia on ambulatory dental patients. O.S., O.M. and O.P. December 1959.

Serial No. NIDR-59 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Investigations into the Etiology of Recurrent Aphthous Ulcerations and Other Oral Nonmalignant Soft Tissue Diseases.

Principal Investigator: Dr. I.I. Ship

Other Investigators: None

Cooperating Units: University of Pennsylvania, Dental School

Man Years:
Total: 1½
Professional: ½
Other: 1

Project Description:

Objectives:

1. To study and isolate the various external and environmental etiological factors involved in the pathogenesis of recurrent aphthous ulcerations and other nonmalignant, chronic, and recurrent oral soft tissue diseases.

2. To determine the experience and prevalence of recurrent aphthous ulcerations as well as related systemic conditions in a graduate school population.

3. To investigate and elucidate the gross and microscopic pathology of the lesions.

4. To evaluate various new agents for the control and prevention of recurrences of these conditions.

5. To investigate and clarify the multiple diagnostic criteria for this group of diseases.

Part A (continued)

6. To investigate the relationship of adrenal-cortical activity to the formation of aphthous ulcerations and other recurrent oral diseases.

Methods Employed:

1. Examination includes:
   a) Viral cultures and serological antibody testing.

   b) Allergic tests with the utilization of synthetic diets on hospitalized patients.

   c) Hemotological studies.

   d) Eosinophil counts and plasma cortical steroid determination.

   e) In vivo microscopy and microphotography.

   f) Biopsy and pathological examination.

   g) Clinical consultation.

2. a) Administration of questionnaire to 1800 graduate school students at the University of Pennsylvania to determine the incidence and experience of recurrent aphthous ulcerations and recurrent herpes labialis as well as other oral soft tissue diseases.

   b) Active follow-up of this group with examination of past medical history, review of systems, and clinical oral examinations.

   c) The maintenance and review of monthly diary forms recording duration. location, and severity of individual lesions in each patient for a period of 18 months.

3. Experimentation with new forms of topical anesthetic agents with low toxicity and high potency for supportive therapy.

4. Double blind clinical investigations of agents for prophylactic administration to prevent recurrences of aphthous ulcerations. Special diary forms recording location,

Part A (continued)

duration, and severity as well as number of individual lesions during test periods on specific double blind medications provide the criteria for the evaluation of all agents.

Patient Material:

1. Individuals suffering acute, active disease referred from the local community and maintained as outpatients on experimental therapeutic regimes, or admitted as in-patients to the Clinical Center for careful follow-up.

2. Students attending the University of Pennsylvania Schools of Dentistry, Medicine, Veterinary Medicine, and Graduate Nursing.

3. Student nurses in the University of Pennsylvania Nursing School, the Presbyterian Hospital Nursing School, and the Philadelphia General Hospital School of Nursing.

4. Students of the Dental Hygientist School of the University of Pennsylvania.

Major Findings:

1. Tissue culture technics (using monkey kidney, human skin, rabbit kidney, and hela cells) have failed to isolate herpes simplex virus or other viruses from 100 aphthous ulcerations (in various stages of development) in 31 patients. Herpes simplex virus was, however, isolated from 13 of 25 recurrent herpes labialis vesicles in 11 patients.

2. Acute exacerbations of aphthous ulcerations with the formation of multiple acute lesions appear to coincide with periods of emotional stress in many patients. No cause and effect relationship has been demonstrated.

3. In contrast to previous reports in the literature, recurrences in females in the present study did not follow a menstrual cycle. Remission during pregnancy, however, has been demonstrated in six instances.

Part A (continued)

4. There was a failure of regression of lesions or remission of disease when patients were placed on synthetic, non-allergic diets with the elimination of all cosmetics, aromatic oils, and synthetic clothing. Dietary allergy did not have etiological significance in the progression of disease in the 8 patients examined in this manner.

5. Allergic etiology was demonstrated in one case.

6. Hydrocortisone, taken systemically or applied locally to the affected tissues, was beneficial in the prevention of recurrences and the regression of aphthous ulcerations. Analysis of initial results shows a dose-response relationship in most patients, (prophylactic efficiency being related to the level of drugs applied to tissues twice daily).

7. A solution containing 0.5% dyclonine hydrochloride and 0.5% diphenhydramine hydrochloride applied topically to acute aphthous ulcerations gave significant temporary relief from pain, but had no effect on the severity and duration of individual lesions. This anesthetic solution was also demonstrated to have broad clinical effectiveness in many forms of painful stomatitis, including pemphigus vulgarus, leukemia, oral lichen planas, and stomatitis secondary to cancer chemotherapy.

8. 52.5% of males and 57.2% of females in the study reported positive history of recurrent aphthous ulcerations or canker sores. Differences between the sexes were significant (P=.02). However, no differences were noted as regards geographic origin, racial background, age, marital status, school or class. Significant religious group differences were noted.

9. 38.4% males and 38.2% females in this population had a history of recurrent herpes labialis or "fever Blisters." Geographic origin, racial background, age, sex, marital status, school, and class showed no significant group differences. Significant religious group differences were once again noted, but these varied from the group differences seen in the aphthous ulceration category.

Part A (continued)

10. The interrelationships between both diseases were examined in the same group. Comparison of the expected and actual proportion of students with both diseases showed a relationship in females but not in males. Students with recurrent aphthous ulcerations experienced more frequent episodes of disease than students with recurrent herpes labialis. This fact was clearly demonstrated in examinations of the group that had both diseases.

Significance to Dental Research:

Investigations into the many individual and group factors in these diseases have led to increased information regarding their interrelationships in a population group, and could contribute to a better understanding of etiology and subsequently to a rational method of treatment.

Proposed Course of the Project:

To continue the experiment in progress. Examination of metabolic and enzymatic relationships within tissues prone to disease in affected patients will be initiated. Continuation of testing of agents for prophylaxis and supportive care of patients with recurrent aphthous ulcerations is planned. Examination of other oral soft tissue nonmalignant diseases will be expanded.

Part B included: Yes

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Ship, Irwin I., D.M.D., Williams, Anderson F., D.M.D., and Osberoff, Boris J., B.S.: The development and clinical investigations of a new oral topical anesthetic (Dyconine Hydrochloride-Diphenhydramine Hydrochloride) for acute and chronic oral lesions. Accepted for publication in the Journal of Oral Surgery, Oral Medicine, and Oral Pathology.

2. Driscoll, Edward J., D.D.S., Ship, Irwin I., D.M.D., Baron, Samuel, M.D., Stanley, Harold R., D.D.S., and Utz, John P., M.D.: Chronic aphthous stomatitis, herpes labialis and related conditions. Combined Clinical Staff Meeting of the National Institutes of Health. Annals of Internal Medicine, Vol. 30: 1475-1493, June 1959.

3. Ship, Irwin I., D.M.D., and Ashe, Warren K., B.S.: Recurrent "fever blisters, and "canker sores": Attempts to isolate herpes simplex virus in tissue culture. Submitted for publication.

4. Ship, Irwin I., D.M.D.: The diagnosis and treatment of ulcerations in the mouth: Dental Health Memorandum, U.S.P.H.S., December 1, 1959.

5. Ship, Irwin I., D.M.D., Morris, Alvin W., D.D.S., Durocher, Roy L., D.D.S., and Burket, Lester W., D.D.S.: Recurrent aphthous ulcerations and recurrent herpes labialis. I. Experience in a graduate and nursing school population. Submitted for publication in the Journal of the American Dental Association.

Serial No. [illegible] 60 (c)
1. Clinical Investigation
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Stress in the Dental Patient

Principal Investigator: Dr. Irwin I. Ship

Other Investigators: None

Cooperating Units: None

Man Years (calendar year 1959)	
Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives:

1. To determine and compare changes in adrenocortical activity caused by various aspects of dental therapy in healthy adult humans.

2. To investigate the changes in adrenocortical responses to dental stress induced by the use of local anesthetics and simple barbiturate premedication.

3. To investigate the systolic and diastolic blood pressure responses to various aspects of dental therapy in healthy adult humans.

4. To establish a reliable index of physiological response for future testing in dental techniques and therapeutics.

Methods Employed:

1. All dental treatments are standarized and divided into six categories, interview, radiographic examination, oral examinations, prophylaxis, operative dentistry, and oral surgery.

Part I (continued)

2. Total eosinophil counts taken from capillary blood by standard finger puncture prior to dental therapy are compared with similar counts taken 4 hours following treatment.

3. Examination of minute-to-minute systolic and diastolic blood pressure changes during 40 to 60 minute dental appointments using the Colson Model 110 blood pressure recorder.

4. A 50 milligram pentobarbital sodium capsule, an identical placebo capsule, or no capsule is dispensed by the Pharmacy according to a regimen determined by a statistician and kept blind both from patient and investigator. Capsules are taken ½ hour prior to dental appointments.

5. Two cc of 2% Lidocaine solution, 2 cc of sterile saline, or no injection is made at each appointment according to a schema established by the statistician.

6. Controls for this study consist of a) ordinary dental appointments randomly selected as regards sequence of treatments at which time no procedures are attempted and patient rests for the entire time in a dental chair, b) four eosinophil counts taken at unscheduled times so as to indicate normal daily variations.

Patient Material:

Approximately 25 healthy adult employees of the National Institutes of Health with extensive dental caries referred by the Employee Health Service.

Major Findings:

1. A comparison of stress responses in the various treatment groups with responses in the control appointment groups revealed significant changes in the former.

2. Stress responses to various operative dentistry procedures demonstrated no significant differences when compared with responses to interview, X-ray and oral examinations, or prophylaxis appointments.

3. Local anesthesia had no effect on the stress reactions in operative dentistry.

Part I (continued)

4. Barbiturate premedication was associated with slight reduction in the stress reaction in operative dentistry while placebo had no effect.

5. The stress reactions to oral surgical procedures were greater than that to all other procedures tested. A substantial reduction in stress response in this group was established by barbiturate premedication.

6. Anxiety, fear, and apprehension during the course of the dental appointment appeared to be the primary factor involved in the stress inducing aspects of dentistry.

7. The effects of dental procedures and control of the blood pressure were investigated in 17 healthy adult subjects. Analysis of blood pressure results revealed the following:

a) Fluctuations in the range of 25 mm of mercury were observed around the mean systolic and diastolic pressures. These fluctuations were independent of age, sex, time of day, day of week, and initial blood pressure.

b) The various dental procedures showed insignificant net differences in either mean systolic or mean diastolic blood pressures.

c) Patients appeared to fall into three distinct categories of response to dental procedures and were classified as "nonreactors", "reactors", and "hyper-reactors."

Significance to Dental Research:

Although little is known regarding physiological response to actual dental procedures in human beings, the subject is frequently discussed particularly in reference to newly developed dental instruments and techniques. A physiological tool for the evaluation of techniques and therapeutics in the dental field would be a valuable contribution to the profession.

Part A (continued)

Proposed Course of Project:

1. Further evaluations of the effects of general anesthesia, extensive oral surgery, and prolonged periodontal therapy.

2. Investigations into the responses of individuals with compromised systemic resistance to stress and comparison with normal adults.

3. Concomitant determinations of physiological and procedural variables to determine the mechanisms of the magnitude of activity demonstrated. These will include effects of speed of the dental engine on blood pressure, pulse, respiration, skin temperature, eosinophil count changes, and other physiological variables.

Part B included: Yes

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Ship, I.I., D.M.D., and White, C.L., A.B., Physiological Response to Dental Stress." Accepted for publication in the Journal of Oral Surgery, Oral Medicine, and Oral Pathology.

2. Ship, I.I., D.M.D., The Response of Systolic and Diastolic Blood Pressures to Dental Stress. Accepted for publication in the Journal of Oral Surgery, Oral Medicine, and Oral Pathology.

Serial No. NIDR-61 (c)
1. Clinical Investigations
3. Bethesda, Md

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Secretory Sialography in Patients with Sjogren's Syndrome and Rheumatoid Arthritis

Principal Investigators: Irwin I. Ship and Kurt Block

Other Investigators: Dr. Martin J. Wohl, Dr. Richard Oglesby, Dr. Richard A. Malmgren, Dr. Joseph J. Bunim, Dr. William R. Carroll, Dr. Philip R. McMaster and Dr. Robert M. Stephan.

Cooperating Units: National Institute of Arthritis and Metabolic Diseases.

Man Years (Calendar year 1959) NIDR

Total:	1/4
Professional: (Dr. Ship)	1/4
Other:	0

Project Description

Objectives:

1. To investigate salivary gland physiology and pathology in Sjögren's Syndrome in relation to Rheumatoid arthritis and other connective tissue diseases, and to study the oral structures in these diseases. To document the clinical, serological, immunological, and pathological spectrum of Sjogren's syndrome and related connective tissue diseases.

Methods Employed:

Sjögren's syndrome refers to a complex consisting of keratoconjunctivitis sicca, xerostomia and/or salivary gland enlargement and rheumatoid arthritis or one of the related connective tissue diseases. Dryness of the mucous membranes of the nose, pharynx, and bronchi, esophagus and stomach as well as that of the vagina may occur. Histological examination of the glands responsible for keeping these tissues moist reveals atrophy of acinar parenchyme, intensive lymphocytic infiltration and in the lacrimal and salivary gland, epi-myoepithelial islands.

Techniques for the physiological and pathological evaluation of major salivary glands have been developed and standardized. Secretory sialography is being done on each patient to define qualitative and quantitative gland changes associated with diminished secretory efficiency. Standard saliva collections using the Lashley Cup apparatus as well as gland biopsy followed by pathological evaluation contribute to clinico-pathological correlation, and further extension of our knowledge of these structures.

Clinical Aspects:

Twenty-one female patients with clinical evidence of Sjögren's syndrome were studied until the present time. The diagnosis of Sjögren's syndrome was based on symptoms related to dryness of mucous membranes and symptoms of the related connective tissue disease. Confirmatory examinations and laboratory studies were performed by cooperating units in the Ophthalmology Service (Dr. Richard Oglesby) and NIDR. The diagnosis of keratoconjunctivitis sicca was based on filamentary keratitis demonstrated by biomicroscopy, decreased tear flow demonstrated by Schirmer Test #1, and abnormal staining of the bulbar conjunctiva and cornea by Bengal-rose stain. Dental examination disclosed dryness of the oral mucous membranes, unusual pattern of dental caries in some patients and enlargement of major salivary glands. Parotid flow was measured by use of the Lashley cup and indicated marked reduction or complete absence of salivary flow in several patients in this group. (Dr. Robert M. Stephan, NIDR). On secretory sialography of the parotid gland sialatasis was demonstrated in all of the patients examined. (Dr. Irwin I. Ship, NIDR). Approximately 40 other patients have been examined by these techniques and serve as a control group. According to the American Rheumatism Association criteria for the diagnosis of rheumatoid arthritis the initial group of 21 patients was divided into four groups. In the first group were 8 patients with definite or classical rheumatoid arthritis; in the second group were three patients with possible rheumatoid arthritis; two had scleroderma and 8 patients had only oral and ocular manifestations of Sjögren's syndrome.

Laboratory Examinations:

An unusual finding in this group of patients was a combination of low fixed urinary specific gravity and low blood urea nitrogen. This may be related to the chronic intake of large amounts of water. (This phase of our

study is being conducted by Dr. Sheldon M. Kahn-NHI). Six of these patients had low white blood cell counts and many had eosinophilia. Two patients had thrombocytopenia without purpura. The erythrocyte sedimentation rate was elevated in 20 of 21 patients.

Serum Protein Changes:

Total serum proteins were less than normal in the first three groups of patients and were increased in the patients in group four. Concentration of serum albumin was below normal in the entire group. Serum globulin concentration was increased in all four groups; a marked increase was noted in the gamma globulin concentration, especially in the last group.

Serological Reactions:

The bentonite flocculation test (BFT) was positive in 20 of 21 patients and the sensitized sheep cell agglutination test was positive in 18 of 20 tested. The serum component responsible for the positive BFT was located in the bottom fraction or pellet obtained by ultracentrifugation through a sucrose density gradient. Sera from the four groups behaved similarly. The serologically active fractions contained less than 10% of the total protein used in each experiment and contained all of the high molecular weight gamma globulin (19S) as determined immunologically by gel diffusion using as antiserum to 19S gamma globulin.

Analytical ultracentrifugation (Dr. William R. Carroll) revealed unusual high molecular weight complexes with a sedimentation constant of approximately 22, only in sera from patients with Sjögren's syndrome and rheumatoid arthritis. (These 22S complexes have been described in approximately 30% of rheumatoid arthritis patients, especially those with very high titers in serological tests for rheumatoid arthritis.) Furthermore, these ultracentrifugation studies indicated that the increase in gamma globulin concentration was due chiefly to an increase in the 7S class of proteins.

Serum constituents with special affinity for tissue nuclei were demonstrated using fluorescent antihuman gamma globulin (Dr. Richard A. Malmgren). Significant titers were obtained with 13 of 21 sera from patients

in this study. In comparison, only 4 of 27 rheumatoid patients without Sjögren's syndrome had significant titers in this technique. However, only two patients had positive L.E. cell preparations; in one of these patients this was associated with clinical evidence of systemic lupus erythematosus.

Five of the patients in this group had positive direct Comb's tests. Studies are proceeding to identify the antibody coating red blood cells in these patients. Three of the patients had antibodies to thyroglobulin coated, tannic acid treated sheep erythrocytes. There was no evidence of clinical thyroid disease in these patients.

Experimental Studies:

Attempts to demonstrate antibodies to saline extracts of human salivary gland have been inconclusive. Preliminary experiments using complement fixation indicated that sera of patients with Sjögren's syndrome reacted with saline extracts and fixed complement. These studies are to be further confirmed and extended.

Attempts to use saline extracts of submaxillary glands as antigen in agar gel diffusion studies also led to inconclusive results.

Experimental Production of Sjögren's Syndrome:

Rabbits were injected with homogenates of rabbit submaxillary glands emulsified in Freund's adjuvant. Rabbits were injected in the foot pads and in the intrascapular region.

These animals were observed for 5 months and no ocular or oral disease was observed. Repeated skin tests with homogenates of salivary gland were negative. Serial bleeding and testing did not reveal precipitating antibodies for salivary extracts. Histologic examination of tissue from two injected rabbits did not reveal significant abnormality in the salivary or lacrimal glands.

Similar experiments have been started in guinea pigs injected with homogenates of lacrimal glands emulsified in Freund's adjuvant. After five weeks these animals showed skin reactivity to homogenates of lacrimal gland. Histologic examination of tissue from these animals is pending.

Part A (Continued)

Significance to Research:

Sjögren's syndrome is a little known variant of rheumatoid arthritis. Preliminary studies suggest that it may be due to an autoimmune process which interferes with the function of glands responsible for moistening various mucous membranes. A better understanding of this syndrome may increase our knowledge of rheumatoid arthritis, related diseases, and salivary gland functions.

Future Experiments:

1. Further attempts will be made to demonstrate antibodies to salivary and lacrimal gland constituents using complement fixation, precipitation, and agglutination techniques.

2. Additional animal experiments will be conducted in an attempt to reproduce Sjögren's syndrome.

3. Clinical studies will continue in order to define the full spectrum of this syndrome, including study of patients with only oral or ocular disease.

4. The renal defect in Sjögren's syndrome will be further defined.

5. Studies will continue to delineate the pattern of dental caries and abrasion seen in patients with Sjögren's syndrome.

6. Studies are planned to investigate the clinical and serological manifestations among the family members with this syndrome.

7. Investigations into the relationships of pathological, secretory, and sialographic changes in the salivary glands will be continued.

8. At present, two groups are being carefully examined in reference to salivary gland functions: patients with Sjögren's syndrome (with rheumatoid arthritis) and a group with rheumatoid arthritis without Sjögren's syndrome. The addition of a third, non rheumatoid arthritis, nor Sjögren's syndrome patients would provide an absolute control for the study.

Part B included: No

Serial No. NIDR-62 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Effect of Dietary Phosphates on Dental Caries in Children.

Principal Investigators: Drs. I.I. Ship and O. Mickelsen

Other Investigators: Drs. F.J. McClure, R.C. Likins, I. Zipkin, A.L. Russell, and Mr. C.L. White (NIH), Dr. H. Schraer (The Pennsylvania State University), Drs. B. Bosley, G.E. Waterman, and Miss H.G. Olson (Division of Indian Health), Misses M. Talcott and B. Wenberg (South Dakota State College of Agriculture and Mechanic Arts)

Cooperating Units: Division of Indian Health; Home Economics Department, South Dakota State College of Agriculture and Mechanic Arts; Department of Physics, The Pennsylvania State University, Bureau of Indian Affairs.

Man Years (calendar year 1959)
Total: 4 2/3
Professional: 3 1/3
Other: 1 1/3

Project Description:

Objectives:

1. To determine whether the addition of phosphates to the diets of children will reduce the incidence of dental caries.

2. To determine whether the addition of phosphates to the diets of children will affect their growth and development.

3. To evaluate the efficacy of added calcium (the other component of the phosphate compound) as a means of reducing the absorption and retention of strontium^{90}.

Part A (continued)

Methods Employed:

1. Addition of 2% $CaHPO_4$ (incorporated in the salt) to bread in four boarding schools and the addition of a placebo (untreated salt) to the bread in four control schools. This will provide approximately 1 gram of added calcium per day for each child. Neither the subjects nor the investigators know the identity of the experimental and control schools. Total number of 7 to 14 year old children in the study is 1800.

2. Examination procedure:

    a. Annual dental examination including bilateral bite-wing radiographs on all children.

    b. Quarterly measurements of height and weight on all children.

    c. Annual bone density determinations using hand radiographs on a sample of 200 children from both control and supplemented schools.

    d. Annual saliva collection and analysis on a sample of 200 children from both control and supplemented schools.

    e. Regular food intake surveys including collection and analysis of representative diets in all schools.

    f. Controlled balance study on a small group of children in a control and a supplemented school for calcium, phosphate, magnesium, and strontium^{90}.

    g. Annual physical examinations for nutritional status on a sample of children in both control and supplemented schools.

    h. Annual blood and urine collection on a sample of children in both groups of schools for hemoglobin (blood), calcium, phosphorous, and magnesium (urine).

Part A (continued)

Major Findings:

1. Preliminary dental survey shows a moderate incidence of caries and periodontal disease among these children.

2. The Indian Children on entering school (five to seven years of age) appear to be markedly underdeveloped in so far as height and weight are concerned. The older children compare favorably with the standards for white children.

Significance to Research:

1. Although fluorine added to drinking water reduces the incidence of dental caries by 50 to 65%, there is still a large increment of caries to be taken care of. There is a public health need to develop a means of reducing the incidence of dental caries among the people who do not have access to a fluoridated water supply.

2. The addition of calcium phosphate to the diet offers an opportunity to evaluate the effects of long-term supplementation on growth and development.

3. The study offers a means of securing an answer to a fundamental public problem; namely, will added calcium reduce the absorption and retention of strontium^{90}.

Proposed Course of the Project:

1. A two to three week balance study will be initiated during the first part of the summer vacation (June, 1960) in selected control and supplemented schools. Repetition of balance studies will be carried out in June, 1961.

2. Continuation of the total study for one and possibly two additional years.

Part B included: No

Serial No. NIDR-63 (c)
1. Clinical Investigations
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on Periodontal Dressings. Part II. A Hydrogenated Fat-Zinc Bacitracin Dressing.

Principal Investigator: Dr. P.N.Baer

Other Investigators: None

Cooperating Units: Dr. Charles F. Sumner III, Chief, Periodontia Section, Walter Reed Army Hospital. Dr. John Scigliano, Clinical Center, National Institutes of Health.

Man Years (calendar year 1959):

Total:	1
Professional:	½
Other:	½

Objectives:

To develop a post-surgical dressing which will afford maximum healing and greater patient comfort.

Methods Employed:

Patients with a diagnosis of gingivitis or periodontitis who required surgery as part of their therapy were used as the subjects. The experimental dressing (Zinc-Bacitracin) was placed over one half the operated area and a control dressing over the other half. Both dressings were removed at the end of one week. In the experimental dressing, a hydrogenated fat was substituted for eugenol. The powders were the same.

Major Findings:

1. No allergic reactions or Candida infections occurred in either the experimental or control groups. These observations are based on a total of 735 cases in which zinc bacitracin was incorporated in the powder portion of the dressing.

2. The experimental dressing was much easier to handle and more comfortable than the control, which contained the eugenol in place of the hydrogenated fat.

3. In one-fourth of the cases which involved extensive exposure of alveolar bone, the healing seemed to be at a more advanced stage at the end of the first week under the experimental dressing.

4. No pitting of acrylic occurred when the experimental dressing was placed against acrylic facings.

Significance to Dental Research:

Post-surgical dressings which permit maximum healing and afford maximum patient comfort are of considerable value in the clinical management of the patient.

Proposed Course of Project:

Continued testing and evaluation of the materials contained in periodontal dressings is contemplated.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Beer, Paul N., Charles F. Sumner and John Scigliano. Studies on a Hydrogenated Fat-Zinc Bacitracin Periodontal Dressing. Oral Surg., Oral Med., and Oral Path. (In press).

Serial No. NIDR-64
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Studies on Periodontal Disease in the Mouse

Principal Investigator: Dr. P. N. Baer

Other Investigators: None

Cooperating Units: Mr. J. E. Lieberman, Biometrics Branch, Division of Research Services.
Mr. Carl White, Epidemiology Branch, National Institute of Dental Research.
Dr. George E. Jay, Jr., National Cancer Institute.
Dr. Walter L. Newton, Laboratory of Germfree Animal Research, National Institute of Allergy and Infectious Disease

Man Years (calendar year 1959)
Total: 1
Professional: ½
Other: ½

Subproject A: Effect of Age, Sex, Cage, Environment and Diet on Incidence and Severity of Periodontal Disease.

Objectives:

The inbred strain of mouse, STR/N was found to be susceptible to periodontal disease. As a result, a series of experiments were designed to answer the following questions:

1. At what age does periodontal disease first manifest itself?

2. Is there a sex difference?

3. Is there a cage factor?

4. Does the physical consistency of the diet appear to be important as an etiologic factor?

5. What are the effects, if any, of high fat diets on periodontal disease?

6. What effects, if any, do fluorides have on periodontal disease?

Part A (continued)

Methods Employed:

Inbred mice, both male and female, of strain STR/N were used. They were all housed in plastic cages and maintained in a similar environment. The animals were divided into groups and fed one of the following diets: Purina Laboratory Chow (pellet form), Ground Purina Laboratory Chow, a synthetic diet similar in composition to Purina Chow, a 57% coconut oil diet, a 57% hydrogenated vegetable oil diet. In addition, groups of mice fed Purina chow and 57% hydrogenated vegetable oil diets were also given NaF, 60 ppm in their drinking water.

Major Findings:

1. The onset of periodontal disease occurred between 30-50 days of age.

2. There was no observable difference in sex nor in the number of animals housed per cage that could be related to the disease process.

3. At 170 days of age, all animals had some degree of alveolar bone loss.

4. A nutritionally adequate diet of a hard physical consistency afforded no protection against alveolar bone loss.

5. A nutritionally adequate diet of a soft physical consistency, or sodium fluoride, 60 ppm, in the drinking water, resulted in a reduction of alveolar bone loss in animals fed either a high fat diet or Purina chow.

Subproject B: Effect of Genetics on periodontal disease.

Objectives:

To determine the effect of genetic factors in the etiology of periodontal disease.

Methods Employed:

First generation, second generation, and backcross progenies were obtained from matings between a resistant strain (DBA/2JN) and a strain which is susceptible (STR/N) to periodontal disease.

Part A (continued)

Major Findings:

1. The backcross STR(STR/DBA) shows the same tendency as the parent strain STR N in being susceptible to periodontal disease.

2. The crosses, (STR/DBA)DBA, DBA/STR F_1, DBA/STR F_2, demonstrated a tendency to be as resistant to periodontal disease as the parent strain DBA/2JN.

3. STR/DBA$_{F1}$, and STR/DBA$_{F2}$, are different from the other two groups.

4. The role of the genetic factor is not simple and clear-cut, but must be considered multifactorial in nature.

Subproject C: The Germfree Mouse and its Conventional Control

Objectives:

To study the extent of the contribution made by the oral flora in the etiology of periodontal disease.

Methods Employed:

The jaws and teeth of germfree and conventional white Swiss mice, one to six months of age, were studied for alveolar bone loss, downgrowth of the epithelial attachment and calculus formation. Both groups of animals were fed the same nutritionally adequate diet.

Major Findings:

1. There were no apparent differences in the periodontal conditions of the animals whether they were raises in a conventional manner or germfree.

2. All conventional and germfree mice showed alveolar bone loss and downgrowth of the epithelial attachment starting at approximately four months of age and increasing in severity with increase in age.

3. Calculus occurred on the crowns of the molar teeth in germfree mice as well as in the conventional controls.

Part A (Continued)

Significance to Dental Research

These studies should contribute to our knowledge of the interaction of diet, genetics, and the oral flora, as they affect periodontal disease.

Proposed Course of Project

To continue the study of dietary influences on periodontal disease.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

1. Baer, Paul N. and J. E. Lieberman: Periodontal disease in six strains of inbred mice. J. of D. Research (in press).

2. Baer, Paul N. and W. L. Newton: The occurrence of periodontal disease in germfree mice. J. of D. Research (in press).

3. Baer, Paul N., Jay, G. E. Jr., and Lieberman, J. E.: Studies on Periodontal Disease in the Mouse. IX. The Effect of Genetics. J. of D. Research (in press).

4. Baer, Paul N. and White, Carl L.: Studies on Periodontal Disease in the Mouse. I. Effect of Age, Sex, Cage Factor, Diet. J. Perio. (in press).

Serial No. NIDR-55 (c)
1. Clinical Investigations
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A

Project Title: Histopathology of the Human Pulp

Principal Investigator: Dr. H.R. Stanley, Jr.

Other Investigators: Dr. H. Swerdlow

Cooperating Units: Dental Department, Clinical Center.

Man Years (calendar year 1959):
Total: ½
Professional: ½
Other:

Project Description:

Objectives: To determine:

1. The healing capacity of normal and diseased pulps following drilling procedures per se.

2. What characteristics of pulp pathology are the result of pressure changes when frictional heat is neutralized.

3. The possible additional irritating qualities of amalgam and silicate restorations compared to zinc oxide and eugenol.

4. The traumatic tendency of hand plugging versus pneumatic plugging of amalgam restorations.

5. The mitotic frequency of the odontoblasts and other less differentiated cells of the pulp.

6. The range of pathologic findings to be expected in the pulp resulting from various degrees of carious involvement.

7. The amount of pathology that might be produced by supercooling of teeth by air-water spray techniques.

8. Those characteristics of pulpal tissue that represent true pathologic alterations from those resulting from autolysis due to inadequate fixation and establish the progressive pattern of autolytic disruption from the earliest, most subtle, microscopic changes to complete destruction of the pulpal tissues.

Part A (continued)

Methods Employed:

The principal method employed is the formalin fixation of extracted human teeth, followed by decalcification in 5% formic acid, paraffin embedding, serial sectioning, and staining with hematoxylin and eosin. Masson's trichrome, Wilder's reticulum stain, Feulgen's reaction, periodic acid-Schiff, toluidine blue, and other special stains are also utilized. The measurement of the remaining dentin thickness is obtained with a micrometer ocular. To establish autolytic changes, some teeth are placed in formalin immediately following extraction either intact, or with their roots cut off at various levels up to the cervical line. Other teeth are permitted to bench-dry for various periods of time ranging from 15 minutes to 24 hours, followed by severance of the crown from the root and placement in formalin to permit rapid and maximum penetration of the fixative. These specimens are then decalcified and processed routinely.

Major Findings:

1. Speeds of 50,000 rpm and over, with instruments utilizing the belt-driven or turbine principle, have been found to be less traumatic to the human pulp than techniques utilizing 6,000 and 20,000 rpm with the conventional ball-bearing handpiece. The value of coolants became more significant at the higher speeds. In the absence of adequate coolants, intermittent grinding was of no appreciable benefit. Preparation time at the higher speeds was of little consequence provided frictional heat was controlled. The combination of high speed, controlled temperature, and light load was conducive to minimal pulpal pathologic alteration.

2. Odontoblasts have been considered incapable of mitotic division. Preodontoblastic cells in the cell rich layer are, however, believed capable of dividing into cells which differentiate into odontoblasts as needed. With the accumulation of a number of specimens revealing mitotic divisions approximating predentin, this phase of pulpal regeneration must be re-evaluated.

3. Some carious teeth present little or no pulpal pathology, whereas, others present severe changes. A survey of extracted carious teeth needs to be undertaken to determine what depth of carious

Part A (Continued)

penetration must be reached and what other factors are required before pathologic alterations appear in the pulp.

4. Coolants, although adequate to prevent burn lesions, did not minimize the inflammatory responses when the operative technique required an applied force above eight ounces. Pressure of itself can induce a pulpal inflammatory response.

5. Clinicians report that patients complain of pain from the coldness resulting from the air-water sprays accompanying the use of the air turbines. Conventional speeds only utilize about 25 cc/min. as compared to the turbines using 35-75 cc/min. It has been shown that water temperature drops 15-20° F. when converted to an air-water spray. If body temperature water is used the expected drop in temperature when converted to a spray would still be confined to the desired range of +20° F. ambient.

6. In the fixation study, many teeth that were bench-dried prior to fixation were better preserved than those placed immediately in formalin following extraction Larger teeth were more difficult to preserve than smaller teeth. Older teeth were more difficult to fix than younger teeth because of the lengthening of the roots by hypercementosis which increased the distance the formalin had to travel to reach the coronal pulp. Also, the diameter of the foramina, usually being smaller in older teeth, hinder the penetration of formalin. Good fixation of pulp tissue can seldom be obtained by placing extracted teeth in toto into fixative.

Significance to Program of the Institute:

1. Contradictory findings in the dental literature leave the profession in a quandary concerning the use of certain operative techniques and filling materials. Much of this confusion apparently has eminated from the lack of appreciation of various investigators for the response of the pulp to operative trauma per se. Pulpal response can fluctuate in incidence and intensity according to the speed and pressure employed in instrumentation, the thickness of remaining dentin, the post-operative extraction period, the type of coolant, and the size of the cutting tool. All these factors must be allowed for before evaluating

accurately the additional irritating properties of both permanent and temporary filling materials, cavity liners, and sterilizing agents. Information derived from such studies will be valuable to the entire dental profession.

2. Establishment of recognized characteristics of autolysis should lessen the difficulty of interpreting microscopic sections of pulpal tissues.

3. Occasionally, dental clinicians and investigators have advocated leaving behind some decay when a pulpal exposure appears likely, this procedure being based on the premise that calcium hydroxide, because of its caustic effect when placed into the cavity preparation on top of remaining caries, will inactivate the caries and stimulate the laying down of irregular dentin. Such a policy can be dangerous since the function of Ca OH, as predicted here, has not been proven and the ethical question is raised as to how much caries can be left behind.

Proposed Course of Project:

1. Since we have shown that high speed techniques are biologically safe for the human pulp, emphasis will now be placed on the added effects of filling materials.

2. Since forces above eight ounces have been shown to increase the intensity of the inflammatory response resulting from cavity preparation, evaluation of hand amalgam pluggers versus pneumatic instruments is in order.

3. The method for determining the amount of force employed in our operative studies, employing the Hamilton Postal Scale was coarse. New and more precise electronic equipment has been developed that permits continuous readings of the applied forces during the entire cutting procedure. Such instrumentation would provide a more direct and accurate recording of these measurements and will be used in further studies of this kind.

4. Criticism has often been directed at pulp studies utilizing human teeth of many different sizes and from individuals of wide age distribution. With the

Part A (continued)

accumulation of sufficient numbers of teeth, attention will be focused on the factors of patient age and tooth size to determine whether or not older or larger teeth can modify the pulpal response with other conditions being equal.

Part B included: Yes

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

1. Swerdlow, H. and Stanley, H.R., Jr. Reaction of the human dental pulp to cavity preparation. 2. At 150,000 rpm with an air-water spray. J. Prosthetic Dentistry, 9:121-131, Jan.-Feb., 1959.

2. Stanley, H.R., Jr., and Swerdlow, H. Reaction of the human pulp to cavity preparation in comparative study based upon histopathological findings produced by eight different operative grinding techniques. J.A.D.A. 58:49-59 May 1959.

3. Swerdlow, H. and Stanley, H. R., Jr., Human pulp response to high speed instrumentation. High Speed and Ultra Speed in Dentistry (Chapter 6) by H. Kilpatrick, W.B. Saunders Co., 1959, p. 173.

4. Swerdlow, H. and Stanley, H.R., Jr. Higher speeds in dentistry. District of Columbia Dental Society J., 34:4-6, June 1959.

5. Stanley, H. R., Jr. Teeth--The forgotten biopsies (THE PROPER HANDLING OF TEETH AS PART OF AN ORAL PATHOLOGY BIOPSY SERVICE). U.S.P.H.S. Dental Health Memorandum, September 15, 1959.

6. Stanley, H.R., Jr. and Swerdlow, H. Biological effects of various cutting methods in cavity preparation (The part pressure plays in the pulpal response). In manuscript form, accepted by the J.A.D.A.

DIVISION OF BIOLOGICS STANDARDS

January-December 1959

The Division of Biologics Standards is primarily responsible for administering the provisions of the Public Health Service Act as they pertain to the control of biological products and for the development of Regulations within the provisions of this Act. The legal description of the products covered by the Act is complex, but in effect it includes vaccines, serums, toxins, antitoxins, and related products, including human blood and its derivatives, which are offered for sale, barter and exchange in interstate commerce of for export.

Development of realistic standards for these products and the exercise of proper control over their safety, purity and potency are the main statutory responsibilities of the Division, but the exercise of these responsibilities in such a complex field is only possible with the support of an active research program of sufficient flexibility to provide the information that is needed to meet problems in this complex field as they emerge. One of the main objectives of the Division has been the development of such a research program based on the premise that the control of biological products can only be successful when supported by a program of research designed to anticipate the requirements of the Division's mission. In addition to providing any information needed in meeting the control responsibilities of the Division, the research program also provides a background of ideas for all workers in this field be they in Government service, prívate industry, or elsewhere and serves as a reservoir of trained and alert scientists who can be called upon when problems arise. Since it is obvious that such calls by their very nature, must be unpredictable and fluctuating, research in cognate fields is encouraged so that the professional staff of the Division may follow some of the leads which are suggested by their work. This is difficult to keep in balance, but all scientists engaged in this program except those who have continuing and full-time administrative responsibilities are encouraged to devote up to one-half of their time on research projects of their own devising, most of these being related to the basic mission of the Division, but many examples of lateral activities can be seen

in the individual reports of program activities. Many of the worthwhile leads actually develop in the course of considering the problems encountered in the manufacture and control of biological products.

A major activity in the administration of the Division is the maintenance of the proper balance between the control activities and the research programs, the latter being supported to the degree that such investigations are required in order to meet the control responsibilities of the Division.

The Division conducts a continuing review of existing regulations for the control of biological products, making revisions as necessary and also developing additional regulations as required for new products.

During the past year the regulations concerning sterility, pyrogenicity as well as establishment standards have been carefully reviewed and reworked through the mechanisms set forth for the establishment of Federal regulations. This is laborious, detailed and time-consuming effort which will have to be continued through the entire roster of biological products.

Close and continuous liaison is maintained with technical representatives of industry. Frequent meetings are held between members of the Division's professional and administrative staff and groups of manufacturers who are concerned with common problems. In addition, as many as 200 conferences are held each year with technical representatives of manufacturers who desire to discuss production and testing programs peculiar to their own organization.

The Division services the Technical Committee on Poliomyelitis Vaccine, the Public Health Service Committee on Live Poliovirus Vaccine, as well as numerous Ad Hoc Committees which are appointed from time to time to consider matters relating to the control of biological prod- products.

As reported last year there were encouraging signs that the overall potency of the poliomyelitis vaccine reaching the market was increasing. This has continued through 1959. A consideration of these findings has lead to increased activity in an attempt to develop a more satisfactory method of evaluating potency. A new reference vaccine has been prepared and at the year's end data were being assembled which would help fix its value with respect to antibody response in monkeys, chicks, guinea pigs, as well as in children. A portion of this reference vaccine was provided for the use of the Biological Standardization Section of the World Health Organization and is being

used along with other selected vaccines obtained from other countries in a rather extensive evaluation of potency methods involving a number of countries---including the U.S.A.

During the latter part of 1958 the Division became interested in the problems of live poliovirus vaccine. The Committee on Live Poliovirus Vaccines, appointed by the Surgeon General in 1958, served as a focal point for this work. During 1959 the Committee held seven meetings--some with potential manufacturers and others with members of the staff of the Laboratory of Viral Products. The Division has become active in examining the factors involved in the concept of a safe and potent live poliovirus vaccine. Working closely with the Committee on Live Poliovirus Vaccines, the Division has turned its attention to such matters as the virulence of candidate strains and the conditions for the conduct of the various tissue culture and serological tests which are needed to assure safety and potency.

A continuing problem for the Division is the acquisition of a balanced professional staff. A number of members have been lost to industry and there continues to be a rapid turnover of the younger medically-qualified people who leave after the required two-year term of required duty. The following additions to the senior staff should be mentioned. These are: Dr. H. M. Meyer, Jr. (Virology), Dr. Harold Baer (Bacteriology-Immunology), Dr. J. N. Ashworth (Biochemistry of Blood), Dr. E. B. Seligmann (Bacteriology), and Dr. J. A. Morris (Rickettsiology). In order to broaden the capabilities and experience of existing staff members, arrangements have been made for one year of training and experience away from the Division as follows:

1959 - 1960 Dr. G. L. Van Hoosier, Jr. - California State Health Department Laboratories in Berkeley (Dr. E. H. Lennette).

1960 - 1961 Dr. Samuel Baron - Medical Research Council Laboratories, London (Dr. Christopher Andrewes).

In the same general effort to broaden the experience and contacts of the scientific staff, Dr. Frank Perkins, a Visiting Scientist for the Medical Research Council Laboratories, London, and who is engaged in virus research related to control of biological products in his home laboratories, is spending a year working in the Division's laboratories.

Members of the staff of the Division attend meetings and work with committees and other advisory groups arranged by such diverse organizations having international activities in this general area as

the World Health Organization, and on a national scale, the National Research Council, the Armed Forces Epidemiological Board, the Council on Drugs of the American Medical Association, the General Committee of Revision of the United States Pharmacopoeia and others. During 1959, Division scientists have taken an active part in the World Health Organization's program for the development of recommendations for the international uniformity of biological products.

During 1959, considerable time continued to be devoted to matters concerned with constructing, equipping, and financing the new DBS building. As the year ends this Division looks forward to occupying its new quarters sometime during July of 1960.

The Board of Scientific Counselors which was established in 1957 meets twice per year and reviews the scientific program of the Division. Such meetins give the senior staff members of the Division an opportunity for describing their projects and particularly for discussing the way in which these integrate with the control responsibilities of the Division as a whole.

The operating functions of the Division are implemented at the present time through the activities of its four laboratories; the Laboratory of Viral Products, the Laboratory of Blood and Blood Products, the Laboratory of Bacterial Products, and the Laboratory of Control Activities. The more important of the activities of these individual laboratories may be summarized as follows:

LABORATORY OF VIRAL PRODUCTS

The Laboratory of Viral Products (LVP), which is the largest of the present four laboratories, in keeping with the Division policy, devotes itself to both control and research projects. Although it is run as two sections; namely, the research section and the testing section, control and research activities are carried on by both.

The main single activity continues to be the control of poliovirus vaccine. One new license was issued during the past year, the first since 1955, and the conferences, examinations, discussions, etc. involved represented a considerable effort on the part of the Division. Two new products containing poliomyelitis antigens were licensed early in 1959, these were multiple antigens containing pertussis, diphtheria, tetanus and poliomyelitis components. These licenses were issued to Merck Sharp & Dohme and to Parke, Davis & Company. Other establishments are interested in similar products. Although poliomyelitis vaccine has now been licensed for about five years, day-to-day

problems continue to arise. With the passage of time there is a tendency on the part of the manufacturers to regard the manufacture of poliomyelitis vaccine as a complex, but now routine procedure. The main problem in this regard is to remain alert and ever watchful, despite the excellent record of the past four and one-half years, in order to assure the safety and potency of the vaccine which reaches the market and to avoid anything untoward which might arise from complacency.

The total program of the laboratory cannot be summarized briefly, but mention of the more sugnificant projects and accomplishments included in the following brief summary arranged according to general subjects will indicate the nature and scope of the program:

Poliomyelitis Vaccine

During the year the following tests were carried out on poliomyelitis vaccine:

Tissue culture safety tests	74
Monkey safety tests	188
Monkey potency tests	47

In addition 55 lots were tested for potency using chicks instead of monkeys.

Studies on the antibody response to poliomyelitis vaccine by man and various laboratory animals such as monkeys, chicks and guinea pigs have been continued with a view to the adoption of a more satisfactory potency test. A test using chicks can be made to work, but collaborative studies carried out in various manufacturing establishments and by the DBS indicate that it is subject to variation from laboratory to laboratory. An important need has been a really satisfactory reference vaccine having known characteristics which correlate with past experience in man and animals. Such a vaccine is now available and recent results suggest that a workable test design should shortly be available.

A large single inoculation (10 ml.) of poliomyelitis vaccine of average or better than average potency was capable of evoking detectable antibody responses within 1-2 weeks following inoculation.

Further investigations on the neutralizing antibody combining test as a method of determining the antigenic properties of inactivated poliomyelitis vaccine has been continued. While the test appears reproducible in the case of individual vaccines, the results

do not correlate very well with those of animal antigenicity tests. For this reason, before the procedure can be recommended for general use in manufacture, it is necessary to carry out further evaluation studies despite the attractive simplicity of the method.

Concentration of poliomyelitis vaccine in the ultracentrifuge was studied with a view to increasing the sensitivity of the monkey safety test. Results with added virus and vaccine containing residual virus indicate the feasibility of the methods and suggest that this procedure could be used for increasing the sensitivity of the test.

Live Poliovirus Vaccine

The ability of attenuated polioviruses to revert to increased virulence is being studied in serial stools from persons given such vaccine in field studies.

Comparison of the neurovirulence for monkeys of the strains of attenuated polioviruses that had been used in field trials done under comparative conditions indicates that while all are capable of producing lesions of poliomyelitis when inoculated intraspinally, definite differences can be demonstrated on intrathalamic inoculation. Under these conditions the Lederle strains show a greater tendency for the spread of histological lesions down the spinal cord. The Sabin strains show this property to a lesser degree, while the Koprowski strains are intermediate in character. These differences in neurovirulence demonstrated by intrathalamic inoculation of monkeys have been reported at the meeting on Live Poliovirus Vaccines held by the Pan American Health Organization last June and have been stressed in the reports of the PHS Committee on Live Poliovirus Vaccines.

Among the markers which may be utilized in the identification of attenuated strains, the K marker has been selected for close study. Attempts are being made to find a test system in which poliovirus strains will be effectively neutralized by homologous sera and heterologous strains will be incompletely neutralized. Two approaches are being made: in the one, the rate of virus neutralization is compared for both the homologous and heterologous systems, whereas in the other, dilutions of virus and serum are made in an attempt to achieve complete neutralization in the homologous system and incomplete neutralization in the heterologous system.

Measles Vaccine

Programs with measles virus and measles vaccine were early

initiated in anticipation that an immunizing agent against measles would soon be available; however, total progress in this area in the country as a whole has been much slower than originally expected. Experience with killed measles vaccine has been discouraging because of low titer virus harvests and poor stability after inactivation, while development of a useful live vaccine has been hampered by the fact that all strains so far tested have caused illness in some of those vaccinated. This illness has not been severe, but it does represent a roadblock in the path to the early availability of a "live" measles vaccine. The main efforts of the Division have been in the development of standard reagents and standard methods. The former pursuit has thus far been rather unprofitable because most of the virus materials obtained from pharmaceutical laboratories - even on a contract basis - have been unsatisfactory due to low antigenicity or instability. Other studies being followed are directed towards the development of standardized serological methods, the preparation of reference sera and the identification of possible "markers of virulence."

Rickettsial Vaccines.

During the past year it has been possible to initiate a limited effort in the area of rickettsial vaccines. This effort, which has been limited by present lack of space, will become more effective with occupancy of the new building. This program has as its first objective the devising of more precise measures of potency for inactivated epidemic typhus vaccine, the evaluation of attenuated E-strain live typhus vaccine, and the development of requirements for Q-fever vaccine.

Respiratory Viruses.

In all, 62 lots of influenza vaccine were tested for potency in 1959. A really satisfactory, rapid potency test is still a great need in the control of influenza vaccine. Efforts to improve and simplify the hemagglutination test for potency continues. While the demand for adenovirus vaccine is less than that for influenza vaccine (only 14 lots of adenovirus vaccine having been submitted for release in 1959) work on different methods of determining potency has continued. One method involving the immunization of guinea pigs and the use of their sera in a serum-virus neutralization test in known numbers of HeLa cells has been studied and shows promise.

Viruses - General.

Ribonucleic acid (RNA) from mouse-virulent and mouse-avirulent strains of poliovirus was studied by injecting it intraspinally into mice. The results suggest that the infective RNA for Type I poliovirus

carries the genetic characteristics which determine the degree of neurovirulence.

Work with tumor materials, including human tumor materials, continues and considerable effort has been expended on the SE polyoma virus. This tumor agent was recovered in tissue culture from AKR mice, and present studies are in the direction of using it as a model for the recovery of a virus or viruses from human neoplasms.

Another tumor agent being studied is the "sarcoma 180" agent. It has been found that mouse sarcoma 180 harbored polyoma virus while tissue culture-passed 180 did not. An interesting observation with the sarcoma 180 agent has been the occurrence of hydrocephalus after 3-6 weeks in about 30 percent of Swiss mice inoculated one day after birth.

Some effort continues to be spent in attempts to cultivate the agent of hepatitis. Tissue culture methods are being used with known infected material.

During the past year an evaluation of the degree of neurotropism of the seed virus used in the manufacture of yellow fever vaccine (17-D strain) was carried out in monkeys. Of 30 monkeys inoculated intracerebrally, 29 showed no signs of encephalitis. Thus, the seed virus comfortably meets the proposed World Health Organization requirements that not more than 20 percent of the test animals shall develop encephalitis. It should be noted, however, that all of the monkeys showed lesions in the brain and spinal cord on histological examination. No lesions were noted in the viscera.

The comparative inactivation characteristics of a number of animal viruses exposed to ultraviolet light indicated that inactivation rate and slope of inactivation curve could be correlated with the individual viruses, but not with their size as in the case with bacteriophage.

An examination of the relative susceptibility of animal viruses to inactivation by the photodynamic action of toluidine blue permits the classification of the viruses studied as follows:

Susceptible	Resistant
T2r+ coliphage	Poliomyelitis I, II, and III
T3 coliphage	
Eastern equine encephalomyelitis	ECHO-1
Vaccinia	Coxsackie A-9 and B-2
Adenoviruses 3, 4, and 7	

Susceptible	Resistant
Simian virus 1	
Rabies	
B-virus	
ECHO-10	
Measles	
Fibroma	
Myxoma	
SE polyoma	

Photodynamic treatment of mixtures of B-virus and poliomyelitis virus destroys all B-virus infectivity (within the limits of sensitivity of the test system) but does not significantly reduce the infectivity of the poliovirus. This process appears to be of practical importance as a possible step in the preparation of live poliovirus vaccines.

A cognate study, but one not related to DBS programs was the determination of the effect of sunlight as a variable affecting the efficacy of sodium pentachlorophenate (NaPCP) as a molluscicide in pools and streams. The findings serve to explain the unexpectedly poor results in certain areas of the world when NaPCP was used in shallow streams in an effort to control the vector of schistosomiasis.

These results on photodynamic inactivation appear to be of considerable practical importance and studies are in progress which are designed to elucidate the mechanism of this process.

Miscellaneous.

Studies were continued on latent infection of stable tissue culture cell lines with L-forms of bacteria with the object of eliminating these microorganisms from the cell cultures. Although a variety of antibiotics and conditions of treatment have been used no permanent solution has been possible.

A semi-automatic diluting machine has been developed for making serial dilutions in serological tests. Evaluation of the machine (fabricated by the NIH instrument section) using a dye indicates that it is capable of accurate dilution in 12 simultaneous operations.

An active factor (or factors) with strong antibiotic action against Staphylococcus aureus and B. subtilis has been demonstrated to be present in abalone juice. This observation is being followed by a study of extracts from other marine creatures.

LABORATORY OF BACTERIAL PRODUCTS

This laboratory continued to operate with a deficiency of staff members during 1959. However, the position of Chief, Section on Allergy and Sensitivity will be filled at the beginning of 1960. Attention will be directed towards the development of specific standards for allergenic products, with emphasis first on ragweed pollen. In this connection there will be liaison with the "Committee for the Standardization of Allergens" which is functioning within the extramural activities of NIAID.

Studies relating to pertussis products, cholera vaccine, Schick test toxin, immunological responses to multiple antigens and of fibroma-myxoma viruses were continued.

The introduction of poliomyelitis vaccine into the well-established triple antigen product, diphtheria and tetanus toxoids combined with pertussis vaccine, has presented a number of control problems which required solution. Due to the fact that changes in manufacturing procedures of the pertussis component in particular were made in order to preserve potency of the poliomyelitis components of the combination, problems in potency, toxicity, stability, and sensitization (mouse) of pertussis vaccine were introduced. In particular, the load of potency and toxicity testing of pertussis vaccine was increased materially. A study relating to the latter factor has been initiated.

During the year considerable progress was made in the development of a reproducible quantitative mouse protective test for potency evaluation of cholera vaccine. With this test, one basis of reference for the study of field trial vaccines will be available. It remains to be determined, however, whether there is a direct relation between potency as judged by such an animal test and human protection. This study along with others on various serological responses to the vaccine and rapid biochemical tests for rapid identification of cholera vibrios are being coordinated insofar as practical with the emerging SEATO Cholera Research Program.

Work on the establishment of a reference diphtheria toxin for the Schick test is coming to an end. There has been a great need for such a reference for a number of years, but its establishment has been a complicated and time-consuming matter. As this particular effort draws to a close, attention is being directed to staphylococcal products -- a group of biological products which have been neglected for some years.

A study of the various media used in the sterility test in

different countries for the detection of the presence of fungi and yeast in biological products and of the optimum temperature of incubation was initiated. Need for additional data was brought out during the revision of the sterility test procedures which were set forth in recent amendments to the PHS Regulations concerning biological products and during participation with a Study Group appointed by the World Health Organization for formulation of international requirements for sterility of biological substances.

The observations on the relation of thermoresistance to virulence among fibroma and myxoma viruses has attracted attention in relation to the general phenomenon of virulence and temperature of propagation, a phenomenon exhibited by polioviruses in the so-called "t marker." The benign fibroma virus is incapable of reproducing above 38°C., but after transformation caused by myxoma DNA, the virus has the characteristics of malignant myxoma virus and is capable of reproducing at higher temperatures.

LABORATORY OF BLOOD AND BLOOD PRODUCTS

The rapid expansion, in the last few years, of the number of licensed establishments* coupled with the increasing complexity of methods and tests employed in production has created a need for some central organization to assist in disseminating the latest information, helping with technician training programs and distributing adequate reference standards. As time and staff are available, this laboratory is working in each of these areas with some success. This almost amounts to an "extramural program" having as its goal the raising of the standards of achievement of the establishments.

The laboratory has begun a program, in cooperation with several national groups, of storing blood of rare and unusual types. This blood is to be available in limited amounts to meet actual transfusion needs. In this way, donors of known rare type can donate at their convenience against remote future needs. In addition, this program offers an excellent opportunity to evaluate under actual operating conditions the best procedures for storing, shipping, and administering blood that has been stored for long periods at extremely low temperatures by a technique developed in this laboratory.

---

* There are now more than 400 operating units involved in the production of blood and blood products.

Also in collaboration with outside groups, this laboratory has accumulated a supply of different bloods of rare types which will be used as reagent cells.

At the request of the Office of Civil and Defense Mobilization dried grouping and typing serum samples from the Civil Defense stockpile were tested to determine their suitability for use after the expiration of the dating period. Although in this case it was not possible as a result of the tests to recommend that the dating period be extended, this type of collaboration with other Government agencies has led to savings in the blood and blood products stockpile.

It is of interest to note that this laboratory is cooperating with the American Standard Association and the International Standards Organization in an effort to achieve standardization of disposable transfusion equipment.

The laboratory research program is designed to obtain information on the properties of blood and blood products which is needed for improvement of existing control procedures or for new tests required for adequate control of new products. The increasing specialization and uniqueness of the new products, particularly in the field of coagulation components, creates a further need for a research program oriented toward the investigation of these products.

During the past year some expansion of the investigation of components of the coagulation system and diagnostic reagents was possible. It is also hoped that it will be possible to expand the cooperative research program on the long-term stability of albumin now being carried out with the Department of Defense and the Office of Civil and Defense Mobilization.

Among the continuing activities of the laboratory are such projects as the following:

> To search for the rare antibodies to the human blood factors which result from the antigenic stimulus associated with pregnancy, blood transfusion and natural exposure and to develop better methods for use in this area.
>
> To establish standards for the antigenicity and viability of the formed elements of the blood. It is interesting in this regard to note that an increasing stock of rare red blood cells is being built up to meet transfusion needs. Such stored cells, after suitable preparation, have been successfully administered in quantity.

To determine the changes which occur in the physical and chemical properties of various human plasma proteins following physical and chemical treatment incidental to processing and to investigate the properties, mechanism of action and assay of blood coagulation components and related systems. The stability measurement of albumin stored for five years shows that definite changes of unknown clinical significance take place at storage temperatures above 5°C. These findings have permitted the extension of the dating period of albumin stored at low temperatures.

To develop improved control procedures and standards by evaluating the present control procedures and manufacturing methods insofar as these affect the safety, purity and potency of biological products derived from human blood.

One rather utilitarian result of some of the interests of the laboratory has been the development of an efficient economical and lightweight shipping container which is now in routine use for the shipping of labile biological materials.

Not the least of the activities and responsibilities of this laboratory is the operation of the Clinical Center Blood Bank. This operation which provides suitable blood, sometimes in considerable quantity, for use in the Clinical Center programs serves as a useful extension of the interests of the laboratory in the standards for blood for transfusion and in research connected with this.

LABORATORY OF CONTROL ACTIVITIES

This laboratory is responsible for the area of activity dealing directly with licensed establishments in relation to the licensing and control of biological products. It is supported by sections on reference standards, on control tests, and on pyrogens. Its activities include:

Determination of eligibility of establishments and of individual biological products for license. This determination is made on the basis of a review of the integrity of management, the physical facilities for manufacture and testing of products, the scientific and professional qualifications of personnel, and the evidence of continued safety, purity, and potency of products for which an application for license is being evaluated.

Supervision of annual and special inspections of licensed manufacturers and of establishments for which an application for license has been made.

Releasing of individual lots of biological products for distribution by manufacturers on the basis of review of manufacturer's and NIH tests, and on other data relating to the safety, purity, or potency of the individual lot of the product.

The establishment and distribution of physical biological standards, reference preparations and control materials.

Review of requirements and Regulations now in effect for such constructive revision as is needed and development of requirements and Regulations needed for new products.

Maintenance of close working relations between this laboratory and the other laboratories of the Division in order to insure continuous knowledge of all activities and data needed for licensing of establishments and new products and of the testing and release of individual lots of products currently licensed.

The variety of scope of the activities carried out by this laboratory may be appreciated by the fact that during the period December 1958-December 1959 a total of 8,220 control tests were carried out within this laboratory to insure the sterility, safety, potency, and purity of biological products.

A summary breakdown of these tests as they pertain to various activities is as follows:

Routine Products for Release................	5,557	Tests
Inspection Samples..........................	1,298	"
Cooperative Service Tests...................	1,063	"
Complaint Investigations....................	46	"
Research....................................	256	"

In addition 885 samples were tested for pyrogenicity.

The data obtained served as the basis of recommendations for acceptance or rejection of the individual lots examined.

During the same period 3,409 lots of biological products were submitted for release by licensed manufacturers. Of these, 3,329 lots

were released, 31 lots were rejected, and 47 lots were withdrawn from consideration of release by the manufacturers.

In order to maintain an adequate supply of physical reference standards for use by the licensed manufacturers in their official control testing, it is necessary to prepare and standardize new liquid lots from the primary dried stocks. The number of lots prepared and standardized during the year were antitoxins - 12, serums - 4, vaccines - 5, toxoids - 4. A total of 134 tests were required to complete a satisfactory standardization of these lots. These tests include flocculation reactions, animal protection tests, animal potency tests, neutralization tests, and a number of specialized tests for specific products.

Standard and reference preparations and cultures are freeze-dried for greater stability during storage. The following were dried between December 9, 1958 and November 30, 1959:

Cultures	924	ampules
Serums	1608	"
Vaccines	832	"
Viruses	998	"

Official standards, reference and control preparations currently maintained include 64 items.

The following standard and reference preparations were distributed to commercial pharmaceutical manufacturing houses, health departments, universities, and foreign countries:

Antitoxins	424
Serums	1993
Vaccines	2963
Toxins	189
Cultures	264
Viruses	254

A culture collection of organisms used in the production and testing of biological products is maintained by this laboratory.

Serial No. DBS-1
1. Office of the Director
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

In the Division of Biologics Standards, program direction and administration is centered in the Office of the Director, with centralization of administrative procedures relating to personnel, travel, supply and accounting. Major objectives are determined and program responsibilities are assigned to the operating units within the Division. For each major area, staffing patterns are developed and are continually reevaluated in relation to program changes. The required financial support is determined for each area in relation to the Division's total budget. A major activity of the Office of the Director is the maintenance of the proper balance between the control activities and the research programs, the latter being supported to the degree that such investigations are required in order to meet the control responsibilities of the Division.

The Director working with members of his immediate staff, including a legal assistant, conducts a continuing review of existing regulations for the control of biological products, making revisions as necessary and also develops additional regulations as required for new products.

Close and continuous liaison is maintained with technical representatives of industry. Frequent meetings are held between members of the Division's professional and administrative staff and groups of manufacturers who are concerned with common problems. In addition, 150 to 200 conferences are held each year with technical representatives of manufacturers who desire to discuss production and testing programs peculiar to their own organization.

The production of poliomyelitis vaccine continues to be closely monitored through the operation of the Technical Aid Program. A liaison scientist, under the direct supervision of the Director, is assigned to each of the establishment manufacturing this product. These individuals routinely visit the industrial plants and maintain close liaison with the technical and research staffs of the producers.

The Office of the Director services the Technical Committee on Poliomyelitis Vaccine, the Public Health Service Committee on Live Attenuated Poliomyelitis Vaccine, as well as numerous Ad Hoc Committee which are appointed from time to time to consider matters relating to the control of biological products.

The Director, or his designated representative, attends meetings of committees and advisory groups of the World Health Organization, as well as those of the National Research Council, Armed Forces Epidemiological Board, etc. During 1959, Division scientists have taken an active part in the World Health Organization's program for the development of international uniformity of biological products.

Within the Office of the Director, the Information Office continues to be concerned with, (1) communication through such media as press, radio, television, publications and exhibits of information to the public on the Division's control and research activities, and (2) internal communication of such information through reports to the Director, NIH, and to the Surgeon General as well as to the Department and to the Congress.

During 1959, considerable time continued to be devoted to matters concerned with constructing, equipping, and financing the new DBS building.

Serial No. DBS 2
1. Control Activities
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Office of Chief, Laboratory of Control Activities

Principal Investigator: Dr. W. G. Workman

Other Investigators: None

Cooperating Units: Three

Man Years:
Total: 3 1/4
Professional: 1
Other: 2 1/4

Patient Days: None

Project Description:

Under the Director, Division of Biologics Standards, this project is responsible for the area of activity dealing directly with licensed establishments in relation to the licensing and control of biological products. This activity is supported by sections on reference standards, on control tests, and on pyrogens.

The activities include:

(a) Determination of eligibility of establishments and individual biological products for license. This determination is made on the basis of a review of the history of management, the physical facilities for manufacture and testing of products, the scientific and professional qualifications of personnel, and the evidence of the safety, purity, and potency of products for which an application for license is being evaluated.

(b) Supervision of annual and special inspections [illegible] manufacturers and of establishments for which an application for license has been made.

(c) Releasing of individual lots of biological products for distribution by manufacturers on the basis of review of manufacturer's and NIH tests, and on other data relating to the safety, purity or potency of the individual lot of the product.

(d) The establishment and distribution of physical biological standards, reference preparations and control materials.

(e) Review of requirements and Regulations now in effect for such constructive revision as is needed and development of requirements and Regulations needed for new products.

(f) Maintenance of close working relations between this Laboratory and the other Laboratories of the Division in order to insure continuous knowledge of all activities and data needed for licensing of establishments and new products and of the testing and release of individual lots of products currently licensed.

(g) Serving on various committees, such as the Council on Drugs of the American Medical Association, the General Committee of Revision of the United States Pharmacopoeia, and collaborating with other official or non-official agencies concerned with the manufacture, testing or control of biological products.

(h) Cooperation with other government agencies particularly the Department of National Defense for interchange of information relating to the control of biological products and their use in preventive medicine.

(i) Maintains contacts with other workers in preventive medicine in this country and abroad.

Significance to Division of Biologics Standards: This project is directly related to the legal and administrative functions of the Division, and requires close liaison with the research activities of the Division in order to put new knowledge to practical use.

Proposed Course of Project: It is proposed to continue the activities of this Laboratory at essentially the present level with increased emphasis on the establishment and use of reference standards since the position as chief of that section has been filled. Improvements continue to be made in control testing procedures. Pyrogen test requirements have been strengthened and it is planned to fill the position [illegible]

Part B included Yes /___/ No /___/

Serial No. DBS 3
1. Control Activities
2. Control Tests
3. Bethesda

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Control Testing and Investigations Related to Control of Biological Products.

Principal Investigator: Mr. Robert W. Kolb

Other Investigators: Mrs. Claire B. Cox
Mr. Otis L. Green
Mr. Harry T. Aylor

Cooperating Units: Lt. Col. Abram S. Benenson, MC, USA
Director, Division of Immunology
Walter Reed Army Institute of Research
Walter Reed Medical Center

Man Years
Total: 10
Professional: 3.0
Other: 7.0

Patient Days: None

Project Description:

The principal objective of this project is to assure the compliance of licensed biological products with standards established under the biologics control provisions of the Public Health Service Act. Individual lots of biological products are recommended for release on the basis of evaluation of manufacturer's and Division of Biologics Standards tests. The evaluation includes control testing for sterility and safety of all biological products and such testing for potency and purity as is required and deemed necessary. The specific products involved include antitoxins, therapeutic immune serums, blood derivatives, bacterial vaccines, toxins and toxoids, viral and rickettsial vaccines, diagnostic substances for dermal tests, antivenins and venoms, and allergenic extracts. Supplemental to control testing are the following activities: (a) evaluations of manufacturer's protocols of tests on lots of biological products for compliance with minimum standards; (b) preparation of releases; (c) maintenance of a permanent record and filing system for releases, protocols of manufacturer's tests, and samples of all biological products submitted to the Division and (d) a continuing reevaluation of methods and operations relative to the establishment of efficient control measures.

In addition to the above activities are a number of research and other functions which have been related to this program as follows: (a) Research dealing with the development of new methods for the control of biological products and designed to improve existing in vivo and in vitro methods of testing; (b) research concerned with the study of stability of biological products and with the development and standardization of new biological preparations; (c) participation in the establishment and product licensing program, which involve the review and screening of new license applications, testing of products submitted in support of such applications, prelicensing inspections and preparation of official reports; (d) participation in the annual inspection program of licensed biological establishments and the control testing of inspection samples collected; (e) cooperative investigations with intra-agency laboratories, commercial establishments, domestic and foreign governmental agencies in which studies and assays are carried out on numerous biological and pharmaceutical preparations; and (f) the orientation and training of new government personnel and visitors from other domestic and foreign health laboratories and institutions in biologic control testing methods and related operations as conducted within the Division.

Major Findings and Accomplishments:

I. Control Testing Activities

(a) During the past 12 months (i.e. December, 1958 through November, 1959) biological products manufactured under U. S. Government license have been tested for conformance to National Institutes of Health requirements and standards. A total of 8,220 control tests were carried out within this laboratory to insure the sterility, safety, potency, and purity of biological products. A summary breakdown of these tests as they pertain to various activities is as follows:

Routine Products For Release..........	5,557	Tests
Inspection Samples....................	1,298	"
Cooperative Service Tests.............	1,063	"
Complaint Investigations..............	46	"
Research..............................	256	"

The data obtained served as the bases of recommendations for acceptance or rejection of the individual lots examined.

(b) A permanent record and filing system for releases, protocols of manufacturer's tests and samples of all biological products submitted to the Division of Biologics Standards was maintained. During the same period indicated above 3,409 lots of biological products were submitted for release by licensed manufacturers. Of these, 3,329 lots were released, 31 lots were rejected and 47 lots were withdrawn from consideration of release by the manufacturers.

(c) Seven complaint investigations of biological products were made during the year. Reports and recommendations for action were made to the Chief, Laboratory of Control Activities.

II. Research and Other Activities:

(a) The cooperative study on the development and standardization of a reference diphtheria toxin for the Schick test, which is being carried out with the Laboratory of Bacterial Products, was continued during the year. On the basis of previous preliminary work a satisfactory toxin was selected for use in preparing a reference preparation. Determinations of the biologic properties such as direct intradermal toxicity, direct lethality, *in vivo* toxin-antitoxin combining reactions and combining velocities in guinea pigs and rabbits have been completed for the toxin preparation. A report of these results has been submitted for review and comment.

Specifications for freeze-drying of the toxin for use as an official U. S. reference have been prepared and requests have been made to obtain cost estimates for completion of the drying process under commercial laboratory conditions.

In addition, a tentative draft on "Additional Standards: Diphtheria Toxin for the Schick Test" has been partially prepared but cannot be completed until the experimental work has been terminated.

Further progress in this problem must be deferred until the dried reference material becomes available. It is then anticipated that studies will continue toward the standardization of the reference in compari-

son to the International Standard for Schick Toxin and with commercial Schick toxins by the proposed MRD and Lr assays in laboratory animals. In these tests significant variations will be determined and a final report will be made.

(b) A collaborative inter-agency project with members of the Walter Reed Army Institute of Research involving laboratory and clinical studies to determine the stability and effectiveness of dried smallpox vaccine has been underway during the past year. This laboratory and Dr. Cutchins of the Laboratory of Viral Products conducted 31 experiments by rabbit scarification and cell culture titration techniques to determine the infectivity of dried and liquid vaccines stored at varying temperatures over a 58 week period.

After 6 months an evaluation was made of the data collected and an interim report was prepared by the Walter Reed workers. The rabbit scarification data at this point showed unsatisfactory potency levels for dried vaccine at +56°, +45°, and +37° C after 2, 8, and 12 weeks storage periods respectively, whereas, dried vaccine kept at +22°, +37°, and -20° C were still regarded as potent after 26 weeks storage. It was interesting to find that comparative assays by other test systems i.e. CAM pock counting, and cell culture titration methods did not completely correlate those shown by the rabbit method. Few human trials were conducted during this period and only limited conclusions could be made from the data obtained.

Currently, a report is being prepared for publication showing comparative stability results by the various test systems after 1 year's study.

As a sequel to the above project, a supplemental stability study was setup for liquid vaccine. A limited quantity of the same dried vaccine, which had been stored at minus 20° C, was restored with its glycerinated diluent, vialed and then samples in the liquid state were placed at -9°, -20°, +4°, +22°, and +37° C. At biweekly intervals the samples were examined for potency by the same test systems indicated above.

The results of the rabbit scarification assays showed the vaccine to be stable at the lower temperature up to +4° C for a 21 week period but at higher temperatures of +22° C and +37° C, potency fell off quite rapidly within the 3rd to 5th and the 1st weeks respectively.

(c) A first draft of a manuscript entitled "A Comparison of the Rabbit Scarification Technique with Titrations in Cell Cultures for the Potency Assay of Smallpox Vaccine", by Robert W. Kolb, Ernest C. Cutchins, Wm. P. Jones, and Harry T. Aylor has been completed in preparation for review by the DBS Editorial Committee. This study was based on an evaluation of commercial smallpox vaccines by the test systems indicated above.

Preparation of a second manuscript entitled "Refinements in the Rabbit Scarification Technique for the Potency Assay of Smallpox Vaccines" by Robert W. Kolb, Claire B. Cox, and Harry T. Aylor, also is in progress. This report is based on recent innovations introduced into the rabbit scarification technique and a statistical evaluation of data obtained over the last 10 years with the DBS Reference Smallpox Vaccine Lot 1b.

(d) The limited study initiated in the latter part of 1957 to evaluate the stability of Dried Rabies Vaccine was concluded during the year. Both commercial and NIH Reference Rabies Vaccine preparations following storage at various temperatures for varying intervals of time, were compared for antigenicity in accordance with NIH Minimum Requirements.

The results indicated that dried rabies vaccine of duck embryo origin remains sufficiently stable to meet the NIH potency requirements for at least six months when stored at 37° C and for at least two years when stored at 5° C. A dried rabies vaccine of rabbit brain origin shows a significant decline in relative potency following storage at 37° C for one month, whereas this product remained relatively stable following storage at 5° C for two years. A few variable results occurred over the two year period which, may well be explained by possible vial variation or certain inconsistencies inherent in the test.

It is recognized that more data are necessary before firm conclusions can be drawn with regard to the relative stability of dried rabies vaccines, particularly of duck embryo origin. Exhaustion of the original material prevented further investigation of this problem. A report of these studies has been prepared and submitted to the files for possible use in future consideration of changes in dating requirements.

This laboratory will continue to assist in the control testing and standardization of future lots of dried rabies vaccine of rabbit brain origin produced as official reference material.

(e) During the current year, numerous new establishment and product applications were submitted for consideration of license. Usually these are referred to committees for prescreening and careful review for compliance with regulations and requirements. This laboratory was involved in a number of such actions during the year. Also, involved is the control testing of the products submitted in support of license application. Complete reports and recommendations were made following review of 11 establishments and 26 product license applications during 1959

(f) Participated in the annual inspection program of biological establishments by carrying out 9 regular and 3 prelicensing inspections. Official reports and recommendations were made on all establishments visited. At the request of the Chief, Laboratory of Control Activities, a proposed schedule of inspections was prepared for next year's visits to all licensed establishments engaged in the manufacture of Allergenic Extracts.

(g) Assisted in the review, modification, and revision of existing regulations and requirements for biological products. Prepared in cooperation with Mr. Fehnel, Laboratory of Blood and Blood Products an initial draft and summary of existing dating requirements for all licensed biological products with suggested revisions.

(h) Twelve visitors representing seven foreign countries were given orientation and training in control testing and related problems during 1959. The length of visitation ranged from half a day to one week.

Proposed Course

The proposed course is to continue the control testing, research and related objectives with increasing efficiency and to reach conclusions on research projects in as satisfactorily and expeditious manner as possible. Also, it is the intent of this laboratory to possibly engage in additional studies as follows:

(1) A collaborative study with Dr. Seligmann, Reference Standards Section, Laboratory of Control Activities, on the standardization of an official NIH Reference Smallpox Vaccine.

(2) A study in cooperation with Walter Reed Institute of Medical Research on the comparative stability of dried smallpox vaccines of egg and calf lymph origins as determined by CAM pock counting and rabbit test methods.

(3) A comparative study of rabbit scarification and rabbit intradermal testing of smallpox vaccines.

(4) Continued study on methods of assay for the fluid diphtheria component of Tetanus and Diphtheria Toxoids (For Adult Use) in order that it may be possible to extend requirements to cover this product.

Part B included Yes / / No / X /

Serial No. DBS 4
1. Control Activities
2. Pyrogens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Section on Pyrogens

Principal Investigator: Mr. Harry W. Burruss

Other Investigators: None

Cooperating Units: None

Project Description:

Complying with the control regulations of the Public Health Service Act, this section has as its primary responsibility the testing of certain licensed biological products to determine their safety, purity, and stability. These biologicals, especially the blood products of human and animal origin, must be pyrogen free; purity is determined in part by total solids content; stability of dried products is measured by moisture determinations. Principal products tested include vaccines, streptokinase, antivenins, antitoxins, blood albumin, globulin, fibrinogen, fibrinolysin, plasma, and serums.

The principal investigator evaluated all pyrogen test result reported in the manufacturer's protocols to the Division of Biologics Standards. On the basis of these evaluations and additional NIH tests on samples of the products submitted, recommendations for release or rejection were made. Records of all tests were maintained and officially reported.

During the year a total of 885 samples were tested for pyrogenicity, which required the use of approximately 2500 rabbits. This figure includes 141 plasma samples from the Laboratory of Blood and Blood Products and 290 pharmaceutical preparations from the NIH Clinical Center Pharmacy. Studies were made to determine the test dose in rabbits on the pharmaceutical products for which no pyrogen test dose had been established.

Ten visitors from domestic and foreign health authorities and from licensed biological establishments were shown at different times the techniques used in performing the work of this section.

Samples of each lot of Normal Human Serum Albumin released by the Laboratory of Control Activities continued to be distributed to various hospitals for clinical testing. Records were kept of the reports received.

Work was completed to find a method to eliminate the thermogenic factor in fresh blood plasma prior to testing this product for pyrogens. If this inherent fever producing factor in fresh plasma is not first eliminated, it is impossible to perform a satisfactory pyrogen test because of the simultaneous temperature rise in the test rabbits due to pyrogens if present and the fever producing factor in the plasma.

In cooperation with Lederle Laboratories a study was completed to compare the pyrogenic sensitivity of two different strains of rabbits used by the two establishments (NIH and Lederle) respectively. This work was undertaken because of difficulties encountered by the manufacturer in obtaining satisfactory pyrogen test results on some lots of their poliomyelitis immune globulin.

The principal investigator continued to serve as a consultant on problems relating to the manufacture and control testing of yellow fever vaccine. During the year six conferences were held with members of DBS Staff and representatives of the manufacturer engaged in producing this product.

Part B included Yes /__/ No /_X_/

Serial No. [illegible]
1. Control [illegible]
2. Reference Standards
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations Related to the Establishment and Maintenance of Reference Standards for Biological Products.

Principal Investigator: Dr. E. B. Seligmann

Other Investigators: Mr. R. P. Miller
Mr. E. C. Russell

Cooperating Units: None

Man Years	Patient Days: None
Total: 5	
Professional: 2	
Other: 3	

Project Description:

Objectives: Since biological products cannot be standardized, with few exceptions, by chemical or physical means, recourse must be had to testing their effects in relation to physical reference preparations. It is the function of this project to provide these standard preparations for the use of manufacturers and others engaged in standardization. In this way uniformity of potency is established throughout the country. It is largely by the use of these standards that those portions of the biologics law relating to potency can be enforced. A great deal of painstaking and responsible work is necessary to insure that standards issued are satisfactory as official standards for this country and bear a known relation to International Standards.

Methods Employed: Fluid preparations must be made and standardized from dried primary standards prior to the expiration date of the current lot. Old standards must be replaced as they become exhausted. Standards must be developed for new products and for certain of the older ones for which it has not been possible to prepare standards heretofore. This is dependent upon advanced knowledge in a number of fields, such as immunology, biology, biochemistry, and medicine. Important features of this work are the improvement of methods of [illegible]

standards, such as by freezing and drying, so that their [illegible] may be maintained, selection or development of standard preparations which will yield clear-cut end points in control testing done by all manufacturers, and correlation of these standards where possible with those established in other countries or with International Standards.

Major Findings and Accomplishments:

(a) In order to maintain an adequate supply of physical reference standards for use by the licensed manufacturers in their official control testing it is necessary to prepare and standardize new liquid lots from the primary dried stocks. The number of lots prepared and standardized during the year were antitoxins - 12, serums - 4, vaccines - 5, and toxoids - 4. A total of 134 tests were required to complete a satisfactory standardization of these lots. These tests include flocculation reactions, animal protection tests, animal potency tests, neutralization tests, and a number of specialized tests for specific products.

(b) Standard and Reference preparations and cultures are freeze-dried for greater stability during storage. This Section dried the following between December 9, 1958 and November 30, 1959:

Cultures	924 ampules
Serums	1608 ampules
Vaccines	832 ampules
Viruses	998 ampules

(c) This Section, from December 9, 1958 to November 30, 1959, distributed to commercial houses, health departments, universities, and foreign countries the following Standard and Reference preparations:

Antitoxins	424
Serums	1993
Vaccines	2963
Toxins	189
Cultures	264
Viruses	254

(d) A culture collection of organisms used in the production and testing of biological products is maintained by this Section. Official Standard, Reference and Control preparations currently maintained are:

1. Standard Preparations

Bacterial Products

Antipneumococcic Serum (Type I, II, V, VII, VIII)
Botulinus Antitoxin, Type A
Botulinus Antitoxin, Type B
Diphtheria Antitoxin
Dysentery Antitoxin (Shiga)
Histolyticus Antitoxin
Oedematiens Antitoxin
Perfringens Antitoxin
Pertussis Vaccine
Opacity Standard for Pertussis Vaccine
Opacity Standard for Typhoid Vaccine
Scarlet Fever Streptococcus Antitoxin
Sordelli Antitoxin
Staphylococcus Antitoxin
Tetanus Antitoxin
Old Tuberculin
PPD of Tuberculin
Vibrion Septique Antitoxin

Viral Products

Antirabies Serum

Blood Products

Thrombin

2. Reference Preparations

Bacterial Products

Botulinus Antitoxin, Type C
Botulinus Toxin, Type A
Botulinus Toxin, Type B
Cholera Opacity Reference
Diphtheria Toxoid (AP)
Diphtheria Toxoid (Fluid)
Diphtheria Flocculating Serum
Haemophilus influenzae Typing Sera,
  Types a, b, c, d, e, f
Histolyticus Toxin
Histoplasmin
Nitrogen Reference
Oedematiens Toxin
Perfringens Toxin
Plague Vaccine
Tetanus Toxoid (AP)
Tetanus Toxoid (Fluid)
Tetanus Flocculating Serum
Typhoid Vaccine
Typhoid Turbidity Reference

Viral Products

Adenovirus Vaccine Types 3, 4, 7
Adenovirus Crude Fluid, Type 3, Strain JF
Influenza Virus Vaccine
Mumps Serum
Poliomyelitis Immune Globulin
Poliomyelitis Serum
Poliomyelitis Vaccine, Type 1, 2, 3
Poliomyelitis Virus, Type 1
Rabies Vaccine
Rabies Challenge Virus
Rabies Production Virus
Smallpox Vaccine
Typhus Serum

Blood Products

Anti-A Blood Grouping Serum
Anti-B Blood Grouping Serum
Anti-Rho (anti-D) Typing Serum
Coomb's Anti-Human Serum
Fibrinogen

3. Control Preparations

Bacterial Products

Antimeningococcic Serum
Hemohilus Influenzae Antiserum, Type b
Scarlet Fever Streptococcus Toxin
Sordelli Toxin
Staphylococcus Toxin
Tetanus Toxin
Vibrion Septique Toxin

(e) Yellow Fever Vaccine manufactured under U. S. Government license has been tested for conformance to the official regulations governing its production. A total of 200 lots were tested for safety, potency, and purity during the year.

(f) A total of 1500 ampules of Reference Rabies Vaccine was prepared and submitted to the World Health Organization for consideration as an International Standard.

(g) A total of 1960 vials of Reference Poliomyelitis Vaccine were prepared and submitted to the World Health Organization for consideration as an International Standard.

(h) Assisted in the control inspection program by conducting three annual establishment inspections during the year.

II. Proposed Course of Project:

It is proposed that the project will be given new support by means of an active research program which will be designed to provide basic data on the properties of existing and new standard preparations. Research will be initiated to develop preparations having greater stability and more precise and points for their titrations. Standards which are badly needed will be developed for products not presently provided with an official standard for the potency test. Potency tests incorporating the use of the standard will then be developed.

III. Proposed Projects:

(a) Standardization of a Smallpox Vaccine for use as an official standard in collaboration with Mr. R. W. Kolb.

(b) Development of a Reference Diphtheria Toxoid for use in the potency test for the fluid Tetanus and Diphtheria Toxoids Combined (For Adult Use).

(c) Studies on the light and dye inactivation of rabies virus in collaboration with Dr. C. W. Hiatt.

IV. Projects in Progress:

(a) Development of an official potency test for Scorpion Antivenin.

(b) Studies on flocculation reactions with diphtheria and tetanus toxins, toxoids, and antitoxins.

(c) Studies on the stability of yellow fever vaccine subjected to various conditions of stress.

(d) Studies on the allergic encephalitic properties of rabies vaccine in collaboration with Dr. G. A. Hottle, Viral Products Laboratory.

(e) Development of a dried Poliomyelitis Vaccine in collaboration with Dr. W. B. Uhlendorf, Viral Products Laboratory.

Part B included Yes /__/ No /X/

Serial No. DBS-6
1. Laboratory of Bacterial Products
2. Section on Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Components of Diphtheria Toxin

Principal Investigator: Dr. Sara E. Branham (Matthews)

Other Investigators: Dr. C. W. Hiatt
Mr. Donald Riggs

Cooperating Units: Biochemistry and Biophysics Unit,
Research Section, LVP

Man Years:
Total: None
Professional: None
Other: None

Patient Days: None

Part B included Yes /X/ No / /

Serial No. DBS-6

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Branham, S. E., Hiatt, C. W., Cooper, A. D., and Riggs, D. B. Antigens Associated with the Toxin of the *Gravis* type of *Corynebacterium diphtheriae*. J. Immun., *82*:397-408, 1959.

Honors and Awards relating to this project:

1959 Medical Women of the Year of the D. C. Chapter of the American Medical Women's Association.

Serial No. DBS-7
1. Laboratory of Bacterial Products
2. Cancer Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Processes of Cell Infection by Virus as Investigated by the Transformation Process and Viral DNAs: A Continuation of the Fibroma-Myxoma Studies.

Principal Investigator: Dr. Lawrence Kilham

Other Investigator: Dr. Joseph Shack (NCI)

Cooperating Units: National Cancer Institute

Man Years:
Total: 3/5
Professional: 1/5
Other: 2/5

Patient Days: None

Project Description:

Objective: The transformation experiments developed over the last three years offer an opportunity for the study of cell infection by virus.

Methods Employed: The methods, in general, are those outlined in the following publications: Kilham, L. et al., Properties of Myxoma Virus Transforming Agent, Proc. Soc. Exp. Biol. and Med., 98:689, 1958 and the one under Part B (Shack and Kilham). Several new methods would include the destruction of viral DNA with nitrogen mustard with preservation of the outer protein, the use of tissue cultures incubated at different temperatures, and further comparisons of cell infection in cells of rabbit and non-rabbit origin.

Major Findings: Myxoma DNA prepared with Urea will result in transformation in tissue cultures of rabbit origin but not in cultures such as squirrel kidney and rat embryo which are of non-rabbit origin.

Significance to the Program of DBS: Knowledge of processes of cell infection and of the activity of viral nucleic acid may well be of fundamental importance to many types of virus work. The situation in regard to tissue cultures of rabbit and non-rabbit origin is of interest in regard to similar studies of Syverton and others on poliovirus in cells of primate and non-primate origin.

Part B included Yes ☒ No ☐

Serial No. DBS-7

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Shack, J. and Kilham, L. Relation of Myxoma Deoxyribonucleic Acid (DNA) to Fibroma-Myxoma Virus Transformation. Proc. Soc. Exp. Biol. and Med., 100:726, 1959.

Kilham, L. The Fibroma-Myxoma Virus Transformation, Advances in Virus Research, VII, Academic Press, New York, New York. (In press)

Serial No. DBS-8
1. Laboratory of Bacterial Products
2. Cancer Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Virus Infection in Relation to Stress--A Study in Relations of Host Factors and Susceptibility to K-virus in Mice.

Principal Investigator: Dr. Lawrence Kilham

Other Investigators: none

Cooperating Units: None

Man Years:
Total: 3/5
Professional: 1/5
Other: 2/5

Patient Days: None

Project Description:

Objectives: The aims of this study are three-fold: (1) to determine factors which limit virulent K-virus infection to suckling mice from 1 - 19 days of age, (2) to test the effect of cortisone, exposure to cold and conflicts among weanling mice on susceptibility to virus. Can one, for example, break down resistance to infection due to age? (3) to find out more about an interesting latent infection, for K-virus commonly occurs in a latent form in cancer in mice and is now being isolated in many laboratories.

Methods Employed: The general methods are outlined in publications as follows: (1) Kilham, L. Isolation in Suckling Mice of a Virus from C$_3$H Mice Harboring Bittner Milk Agent. Science, 116:391-392 (3015), Oct. 10, 1952, and (2) Kilham, L. and Murphy, H. W. A Pneumotropic Virus Isolated from C$_3$H Mice Carrying the Bittner Milk Agent. Proc. Soc. Exp. Biol. and Med., 82:133, 1953. Weanling mice are being kept in a cold room at 4°C. Conflicts are possible when male mice are raised in isolation, then brought together for varying periods.

Major Findings: These are included in the publications on K-virus (see above) and in the somewhat parallel experiments done on the age, immune response, and susceptibility to mumps virus in hamsters.

Significance to the Program of DBS: Research in the basic problems such as thermoreproduction and host factors in relation to virulence and the elucidation of a latent infection. R-virus will grow in adult mice although it causes no illness.

Proposed Course of Project: If results can be established in white mice, an attempt will be made to run parallel experiments in wild mice, which presumably have larger adrenals and can adjust more readily to stress.

Part B included Yes ☐ No ☒

Serial No. [illegible]
1. Laboratory of Bacterial Products
2. Cancer Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Thermo-reproduction in Relation to Virulence of Viruses.

Principal Investigator: Dr. Lawrence Kilham

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 3/5
Professional: 1/5
Other: 2/5

Patient Days: None

Project Description

Objectives: To determine whether a virus adapted to higher temperatures becomes more virulent and whether highly virulent strains become less virulent when adapted to growth at lower temperatures.

Methods Employed: Squirrel virus (SQ) and the Shope fibroma virus will be adapted by slow steps, if possible, to growth at 40°C. or over and myxoma virus to growth temperatures below 30°C. Virulence of all three viruses will be tested in rabbits.

Major Findings: As presented in publication (Part B) Fibroma, a benign virus, has been found to grow at a top limit of 38°C. whereas the virulent myxoma virus can be passed readily at 40°C. These findings relate directly to the types of illnesses induced in rabbits.

Significance to the Program of DBS: The work presented, by invitation, at a conference on Poliovirus virulence was considered by virus experts to be worthy of continuation for the information that it might provide on the problem of virulence among viruses, which is receiving much consideration in relation to live virus vaccines.

Part B included Yes /X/ No /__/

Serial No. DBS-9

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B. Honors, Awards, and Publications

Publications other than abstracts from this project:

Kilham, L. Relation of Thermo-resistance to virulence among Fibroma and Myxoma Viruses. Virology. (In press)

Serial No. DBS-10
1. Laboratory of Bacterial Products
2. Cancer Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Adaptation of Rat Virus (RV) and K-virus to Suckling Hamsters by way of Hamster Embryo Tissue Culture.

Principal Investigator: Dr. Lawrence Kilham

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 3/5
Professional: 1/5
Other: 2/5

Patient Days: None

Project Description:

Objectives: K-virus is being isolated from cancer mice in many parts of the country; Rat Virus (RV) has many similarities to the cancer-inducing Polyoma virus. There is a question whether either of these two agents, K-virus or RV, might not prove to be carcinogenic if present in a suitable host.

Methods Employed: Techniques similar to those used by investigator in adapting mumps virus to suckling mice will be used. Hamster embryo cultures will be grown at temperatures ranging from 36°C. to 40°C.

Significance to the Program of DBS: It is becoming of increasing importance to know and understand viruses which are of natural occurrence in mice as these animals are studied more and more intensively in regard to virus-induced neoplasms. The same reasoning applies to rats. Present experiments are being made in an attempt to reveal whether these two agents are actually carcinogenic.

Part B included Yes / / No /X/

Serial No. [illegible]
1. Laboratory of Bacterial
2. Cancer Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Vertical Transmission of Latent Viruses in Rats, Mice and Hamsters.

Principal Investigator: Dr. Lawrence Kilham

Other Investigators: None

Cooperating Units: None

Man Years: Patient Days: None
Total: 3/5
Professional: 1/5
Other: 2/5

Project Description:

Objectives: To determine whether virus infections can be transmitted from one generation to another by way of natural infection of embryos in pregnant animals.

Methods Employed: (1) Passage of rat and hamster embryo tissue cultures with attempts to neutralize CPE with sera from maternal animals.

(2) Removal of viscera from embryos after dipping whole animal in iodine solution to destroy possible maternal virus on the outside.

(3) Inoculation of pregnant mice with H virus, then subsequent removal of young for testing by inoculation of suckling [illegible]

Major Findings: Viruses from embryos of rats may have been isolated but definite proof will be difficult in absence of [illegible] pathogenicity. The Rat Virus (RV), previously reported (Part [illegible] is possibly transmitted via the embryo.

Significance to the Program of DBS: Latent infection and virus transmission from generation to generation are among the fundamental problems which have to deal with in [illegible] and possibly cancer.

Part B included Yes /X/ No /__/

Serial No. DBS-[illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards and Publications

Publications other than abstracts from this project:

Kilham, L., Oliver, L. J. A Latent Virus of Rats Isolated in Tissue Culture. Virology, 7:428, 1959.

Serial No. DBS-11
1. Laboratory of Bacterial [illegible]
2. Homophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Pertussis Vaccine.

Principal Investigator: Dr. Margaret Pittman

Other Investigators: Miss Emmy Lou Denny

Cooperating Units: None

Man Years: Patient Days: None
Total: 2.3
Professional: 1.4
Other: .9

Project Description:

Objectives: The objectives are that all lots of products containing pertussis vaccine processed by licensed manufacturers have adequate and stable pertussis potency and be relatively free from untoward reactive substances.

Methods Employed: The same as given in 1958 Report, Serial No. DBS-10.

Major Findings: (1) Variations in potency and toxicity of lots of vaccines submitted indicate need to maintain constant surveillance on these properties, especially in multiple antigen products.

(2) Five lots of vaccine from U.S.S.R. were found to have high protective potency, and two lots from Yugoslavia, identical except for presence of adsorbent in one, also were found to have good potency. The adsorbed vaccine was the more potent. Preliminary data from a Yugoslavian field trial indicate a correlation between the protection in children and the protective activity measured in the mouse.

Significance to the Program of DBS: (1) The surveillance of pertussis vaccine provides information relating to its control and improvement.

(2) International contacts and testing of foreign vaccines provide information on status of potency of international vaccines, on methods for improvement, and on relation of mouse measured potency to human protection activity which cannot be obtained in U. S. because of low incidence of pertussis.

Proposed Course of Project: To continue along the same lines.

Part B included **Yes** ☒ **No** ☐

Serial No DBS-11

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Pittman, M. Variations du Pouvoir Protecteur des Differents Vaccins Anticoquelucheux: Leur Rapport avec la Protection de l'etre Humain. Rev. Immunol., 22:308-322, 1958. (Presented at a Seminaire sur la Coqueluche, Centre International de l'Enfance, Paris, France, December 1957.)

Serial No. LBS-[illegible]
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Specific Sensitization of Pertussis Vaccine

Principal Investigator: Dr. Margaret Pittman

Other Investigator: Dr. John C. Feeley

Cooperating Units: None

Man Years: Patient Days: None
Total: 0.3
Professional: 0.2
Other: 0.1

Project Description:

Objectives: To determine the cause for the variations, from [illegible] to marked, in property of lots of vaccines to induce sensitization in mice, manifested by slow anaphylactic death following a second injection and to determine, if possible, its significance in relation to reactions induced in children.

Methods Employed: (1) Sensitizing property of all lots submitted for release is determined by injecting mice with a second dose 14 days after injection in the official freedom-from-toxicity test.

(2) Manufacturers' records of preparation of lots found to be sensitizing and non-sensitizing are being examined in detail to try to find a cause for the variation.

(3) Investigation of the influence of the presence of other antigens is under consideration.

Major Findings: Variations in sensitizing properties have been found to occur not only in lots from different manufacturers, but from a single manufacturer. The vaccine containing one or more antigens may be plain or contain an adsorbent. The highest incidence occurs in multiple antigens.

Significance to the Program of DBS: If the reactions manifested in mice are related to reactivity in children, the finding of the cause for its presence and its elimination from pertussis vaccine would improve the product.

Proposed Course of Project: To continue along the lines given above.

Part B included Yes ☐ No ☒

Serial No. DBS-13
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Sterility Testing

Principal Investigator: Dr. Margaret Pittman

Other Investigator: Miss Emmy Lou Denny

Cooperating Units: None

Man Years:
Total: .5
Professional: .5
Other: .0

Patient Days: None

Project Description:

Objectives: To provide additional information pertinent to the establishment of requirements for detecting the presence of microbial contamination in biologic products both nationally and internationally. Participation in a World Health Organization Study Group on international recommended requirements revealed a lack of comparative data on the growth promoting properties of various media being used in different countries for the detection of the presence of both bacteria and fungi and yeasts. Present study is limited to a comparison of media for growth of fungi and yeasts.

Methods Employed: (1) Background information was obtained through participation with WHO in formulation of recommended requirements for sterility and with DBS in revision of U. S. Regulations for sterility testing.

(2) Media representative of those being used by different nations are being compared for growth promoting properties for fungi and yeasts at different temperatures.

Major Findings: None. Work was started very recently.

Significance to the Program of DBS: Results should provide information that would serve as a basis for the selection of the best

media and temperature of incubation for detecting the presence of fungi and yeasts in biologics.

Proposed Course of the Project: To complete the study as outlined above and to publish the findings.

Part B included Yes / / No /X/

Serial No. [illegible]
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Methods of Preparation of Formalinized Red Blood Cells.

Principal Investigator: Dr. Louis Csizmas

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1
Professional: 1/2
Other: 1/2

Patient Days: None

Project Description:

Objectives: The objective of this project was to develop a method of preparation of stable formalinized red blood cells, that may substitute fresh ones in immunological methods.

Methods Employed: As outlined in 1958 Report.

Major Findings: (1) The theoretical model developed during the elaboration of the method was supported by experiments on the individual factors. High dilution alone enables acceptable preparations, so the interaction of cells and resulting impurities are the main cause of clump formation and deformation. The most important impurity is the hemolysate resulting during the procedure. Hemolysate alone readily forms gel or clumps, when formalinized. Shaking helps eliminate these clumps, by a defibrination-like process, and helps rounding out cells.

(2) Bis diazotated benzidine coupling of antigens [illegible] parallel with tannic acid treatment, and on a similar way. It resulted also in good preparations, but no advantage was seen.

(3) As a major cause of aspecific hemagglutination [illegible] precipitate formation of sera was found. Stabilizers were tested

inhibit this. Small amount of heparin was found to be effective.

Significance to the Program of DBS: Helps to elaborate more reproducible and specific hemagglutination tests.

Proposed Course of the Project: Elaboration of inhibition and double inhibition tests. Use of sensitized cells in standardization for measuring antibody and antigen titers.

Part B included Yes /X/ No / /

Serial No. [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project.

Csizmas, L. Preparation of Formalinized Erythrocytes. Proc. Soc. Exp. Biol. & Med., (In press).

Serial No. [illegible]
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Effect of Combining Antigens and of Aluminum Adjuvants on the Antigenicity of the Individual Antigens.

Principal Investigator: Dr. Louis Csizmas

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1
Professional: 1/2
Other: 1/2

Patient Days: None

Project Description:

Objectives: The trend using multiple antigens and adjuvants in vaccines raises the question of their mode of action and interaction as well as the validity of the potency tests in these complicated situations. The objective of this project is to investigate these problems in order to approach to a better standardization procedure.

Methods Employed: (1) Mouse protection tests with different combinations of diphtheria, tetanus and pertussis antigens and aluminum adjuvants.

(2) Improvement of test methods.

(3) Mathematical analysis of related problems.

Major Findings: (1) The immunizability and adjuvant effect is different in different mouse strains, and so relative potencies of vaccines of different composition determined in a mouse strain may not be valid even for another mouse strain.

(2) The "host" modifying activity of the aluminum adjuvants and pertussis proved to be of minor importance under the conditions investigated.

Serial No. DBS-11
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Re-evaluation of Requirements for Cholera Vaccine.

Principal Investigator: Dr. John C. Feeley

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.6
Professional: 0.8
Other: 0.8

Patient Days: None

Project Description:

Objectives: This project has as its objective the investigation of methods for potency evaluation of cholera vaccine and the establishment of an improved U. S. Standard of Potency. The study will, insofar as possible, be coordinated with the project World Health Organization's international cooperative efforts toward establishment of a laboratory standard of potency for this product that will reflect protective activity in man.

Methods Employed: Major emphasis has been placed on standardization of technical details related to the performance of quantitative potency tests using mice. Groups of mice are immunized with graded doses of cholera vaccine then challenged after a suitable interval with a constant lethal dose of cholera organisms suspended in gastric mucin. From the results, the median effective dose (ED_{50}) is determined, and the relative potency determined in relation to a reference vaccine.

Freeze-dried monovalent Ogawa and Inaba type cholera reference vaccines are being established.

Attention is also being given to determining the serological response of animals to cholera vaccines measured as agglutinins and

(3) An improved variation and mathematical analysis of the Ouchterlony gel diffusion method was elaborated. Mathematical analysis of "equivalent precipitating zones" resulted in new points.

(4) The methods applied for the simultaneous diphtheria and tetanus potency tests of vaccines in mice were experimentally established for publication.

(5) Elaborating the analysis of "slopes" by the Spearman method exact and unbiased estimates were found for the variance of the variance of the binomial distribution.

(6) Optimal weighing of pools of relative potencies and a simplified method of finding their confidence limits was elaborated.

(7) The logical statistical aspects of setting up a standard in general was considered.

Significance to the Program of DBS: The problems solved and partially solved may have application in the standardization of different products, especially of diphtheria and tetanus vaccines.

Proposed Course of Project: To introduce adsorbents (aluminum pertussis) in the gel diffusion systems in studies of mechanisms involved. To prepare the pertinent information for publication

Part B included Yes /__/ No /X/

Serial No. DBS-17
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Schick Test Toxin (Diphtheria)

Principal Investigator: Dr. Michael F. Barile

Other Investigators: Mr. Donald B. Riggs
Mr. Robert W. Kolb

Cooperating Units; Section on Control Tests, LCA

Man Years:
Total: 1.4
Professional: 0.7
Other: 0.7

Patient Days: None

Project Description:

Objectives: (1) To select a diphtheria toxin for the U. S. Standard for the Schick test toxin.

(2) Re-evaluate the requirements of the Schick test toxin with reference to the selection of a more accurate potency assay

Methods Employed: Determination and characterization of the biologic properties of two toxin preparations have been made, and include direct intradermal toxicity, direct lethality, in vitro toxin antitoxin, combining ability and combining velocities in guinea pigs and rabbits. The results of these determinations have been submitted for review and comment in the last report

In vivo and in vitro determinations of toxin neutralization are being compared from the same host. The number of Schick test toxin doses neutralized by intradermal challenge is compared to amount of circulating antitoxin, as determined by in vitro toxin neutralization.

Specifications for freeze-drying of the toxin for use as an U. S. Standard have been prepared and requests have been made to obtain cost estimates for completion of the drying process by commercial laboratories.

bactericidal, and mouse protective antibodies.

Major Findings: Results to date indicate that a quantitative active mouse protection test is a technically feasible procedure. Some of the experimental conditions of this test have been worked out, but further studies of variables involved are being pursued.

Recently, one version of the mouse protection test was employed in a preliminary investigation of potency of several cholera vaccines used during an epidemic of cholera in Thailand. Monovalent vaccines of a U. S. manufacturer were employed as "working reference" vaccines. These foreign vaccines were fairly comparable in potency to the U. S. products according to the assay procedure used.

Studies on the evaluation of antibody responses in animals as indicators of vaccine potency have not yet progressed sufficiently to warrent comment at this time.

Significance to the Program of DBS: Asiatic cholera continues to be a matter of international public health significance, particularly in the underdeveloped areas of Southeast Asia. Even though exacting scientific data on its effectiveness are not presently available, cholera vaccine is a widely employed control measure, especially during epidemics, and is required for vaccination for travel in these areas.

The World Health Organization envisages the carrying out of a controlled field trial of cholera vaccine at some future date, and the particular vaccines employed in this trial will be subjected to a collaborative study using different procedures in different laboratories throughout the world. Only when comparative data become available on vaccines studied both in the field and in the laboratory can efforts toward establishment of a standard of potency related to human protection be fulfilled.

Proposed Course of the Project: Work completed to date will be extended to cover the various technical aspects of potency tests on cholera vaccines with the anticipated goal of establishing adequate and reproducible laboratory methods for this purpose. These procedures can then be applied to the assay of vaccines employed in field trials with the eventual hope of determining which, if any, of these laboratory procedures reflects potency of a vaccine in man.

Part II included Yes ☐ No ☒

Serial No. DBS-18
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: L Forms of Bacteria from Tissue Culture.

Principal Investigator: Dr. Michael F. Barile

Other Investigators: Mr. Donald B. Riggs
Mr. George D. Gardner

Cooperating Units: Tissue Culture Unit, LVP

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days: None

Project Description:

Objectives: To develop methods of purifying the LVP tissue culture lines contaminated with pleuropneumonia-like organisms (PPLO) and/or L forms of bacteria.

Methods Employed: Appropriate media and environment for the cultivation of PPLO and L forms of bacteria was used.

Major Findings: As previously reported tissue culture lines were found infected with PPLO and/or L forms of bacteria. Preliminary tests showed that these organisms are completely compatible with poliomyelitis Type II virus in primary kidney cell cultures, and do not effect the growth rate of this virus.

Proposed Course of Project: To determine the effect of chemical and physical factors, including antibiotics, on clearing tissue lines of L forms. In addition, to determine whether these organisms can be differentiated by serologic procedures.

Part B included yes ☐ No ☒

A tentative draft on "Additional Standards: Diphtheria Toxin for the Schick Test" has been partially prepared, but cannot be completed until the experimental work has been terminated. Further progress in this problem must be deferred until the dried reference material becomes available.

Major Findings: The biological characterization of the proposed U. S. Standard of Diphtheria Toxin for the Schick Test has been presented in the last report. Progress has been made to have the Standard Toxin dried by commercial freeze-drying apparatus.

Significance to the Program of DBS: The establishment of a Standard Schick test toxin (diphtheria) and more precise method of assay will provide means for increasing the quality of the Schick test toxin.

Proposed Course of Project: After the toxin is dried the U. S. Standard will be compared with the World Health Organization Standard Schick Toxin (diphtheria) and possibly with the standards from other countries.

Part B included Yes / / No /X /

Serial No DBS-19
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Filterable Forms from the Human Dental Pulp.

Principal Investigator: Dr. Michael F. Barile

Other Investigators: Dr. Abe Sheingorn

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days: None

Project Description:

Objectives: To establish the incidence of PPLO and/or L forms of bacteria in acute serous pulpitis and to determine the possible significance of filterable forms in human dental pathology.

Methods Employed: Appropriate media and environment for the cultivation of PPLO and L forms of bacteria were used.

Major Findings: A pure culture of a filterable form demonstrating morphological properties typical of PPLO was isolated from the pulp of a patient with acute serous pulpitis.

Significance to the Program of DBS: To establish the natural distribution, habitat, and possible pathogenesis of these organisms for man.

Proposed Course of Project: The project has been terminated.

Part B included Yes ☒ No ☐

Serial No. DBS-19

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Barile, M. F. and Sheingorn, A. The Isolation and Cultivation of a Filterable Form Resembling PPLO from the Human Dental Pulp--A Case History. Oral Surgery, Oral Medicine and Oral Pathology (In press).

Serial No. DBS-20
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Staphylococcal Products.

Principal Investigator: Dr. Michael F. Barile

Other Investigators: Mr. Donald B. Riggs

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days: None

Project Description:

Objectives: To re-evaluate and develop reliable procedures for the evaluation of potency, purity, and safety of biologic products derived from *Staphylococcus aureus*.

Methods Employed: *In vivo* and *in vitro* immunological methods will be used in studying the activity of staphylococcal products including phages for human administration.

Significance to the Program of DBS: In view of the renewed interest in staphylococcal products for both immunization and therapy, the present study will provide background information for the re-evaluation of the present requirements for the currently licensed products and for the evaluation of new products that may be developed.

Part B included Yes ☐ No ☒

Serial No. DBS 21
1. Laboratory of Viral Products
2. Tissue Culture Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Tissue Culture Preparation and Tissue Culture Testing of Killed Virus Vaccines.

Principal Investigator: Dr. Paul Gerber

Other Investigators: Mr. George Gardner

Cooperating Units: The monkey safety testing unit provides monkey kidneys.

Man Years:
Total: 9.5
Professional: 0.5
Other: 9.0

Project Description;

Objective: To prepare primary and continuous cell cultures for research and test purposes and to test vaccine for safety by tissue culture methods.

Methods: The methods are those outlined in the Regulations and tissue culture methods reported in scientific literature.

Major findings:

1. During the year 74 samples of poliomyelitis vaccine and 12 samples of adenovirus vaccine were tested for safety in tissue culture.

2. From January 1, 1959 to December 1, 1959 the following cell cultures were prepared:

a) Primary cell cultures preparations. See attached.
b) Stable cell culture preparations. See attached.

Part B included: Yes / / No /X /

Annual Report For January 1, 1959 - December 1, 1959

Type of Culture	Tubes	2 oz.	MDB	32 oz.
HEP II	8,507	1,529	1,022	58
Human Amnion	16,006	2,477	92	312
HeLa	8,812	2,046	16	108
Monkey Stable	1,705	1,842	-	8
Chang s Liver	816	1,018	5	-
Henley's Intestine	-	226	-	-

Remarks:

MDB=Milk Dilution Bottles

Date 12/3/59

Annual
Primary Culture Production

Annual Report for: January 1, 1959 - December 1, 1959

Culture Productions:

Type of Culture	Tubes	2 oz.	MDB	32 oz.
Monkey Kidney - Rhesus	64,846	80,860	10,572	4,053
Monkey Kidney - Patas	530	977	77	-
Rabbit Kidney	25,238	17,135	146	271
Monkey Heart	50	14	-	-
Rat Embryo	-	-	-	141
Mouse Embryo	843	100	6	29
Hamster Embryo	242	-	-	-
Mouse Kidney	143	15	-	-

No. & Type Kidneys Processed	Vol. Packed Cells
Monkey Kidney - Rhesus-2,334	4,996 cc
Monkey Kidney - Patas- 14	39.5 cc
Rabbit Kidney - 718	1,475 cc
Monkey Heart - 2	1.4 cc
Rat Embryos - 460	52 cc
Mouse Embryos - 367	57 cc
Hamster Embryos - 20	2 cc
Mouse Kidney - 14	3.2 cc

Remarks:

MDB= Milk Dilution Bottles

Serial No. DBS 22
1. Laboratory of Viral Products
2. Tissue Culture Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Experimental Studies with Cell Cultures

Principal Investigator: Dr. Paul Gerber

Other Investigator: Mr. George Gardner

Cooperating Units: None

Man Years:
Total: 1.5
Professional: 0.2
Other: 1.3

Project Description:

(Sub Project A) - Preparation of stable cell lines by freezing.

Objectives: Other investigators have demonstrated that the viability of mammalian cells could be preserved using rigidly controlled conditions of freezing in a special, rather expensive apparatus. This study was designed to develop a simpler, less expensive method for freezing and preservation of cells.

Methods employed: Continuous cell lines derived from human monkey and rabbit tissues were suspended in growth medium containing various concentrations of glycerol. The material was sealed in ampoules, allowed to freeze slowly at -30°C, and was then stored on dry ice at -60° to 70°C. At various time intervals 3 samples of each cell line were tested for viability.

Major findings: Each of 10 different cell lines stored in the frozen state remained viable for at least 14 months.

Significance to Division: Establishment of a frozen cell culture collection eliminates the time-consuming task of maintaining stock cultures and the accompanying risk of contamination by other cell lines or by microorganisms.

Proposed course: If adequate storage facilities become available this project will be expanded to include most continuous cell lines useful for virological studies.

(Sub Project B) - 'Transformation" of primary cell cultures.

Objectives: Several investigators have observed the emergence of clones of altered cells during prolonged growth of primary cell cultures from rabbit kidney, guinea pig kidney, liver and spleen. These "transformed" cells showed a change in morphology, growth characteristics and became sensitive to polio virus infections in contrast to the parent cultures, which were uniformly resistant to poliovirus. The purpose of this study was to repeat these observations under rigidly controlled experimental conditions.

Methods employed: Primary cultures of rabbit kidney, guinea pig kidney, spleen and liver from embryos and adult animals were propagated in various media, subcultured and observed for periods up to 4 months. The conditions employed were such as to minimize the hazard of accidental contamination with continuous cell lines. At various intervals the sensitivity of these cultures to poliovirus was determined.

Major findings: None of the primary cultures of their progeny gave rise to "transformed" cell clones reported by others are the result of rare mutations or accidental contamination by continuous cell lines carried in the laboratory.

Proposed course: For the present, no further work is planned.

(Sub Project C) - Studies on latent infections of stable cell lines with L-forms of bacteria.

Objectives: To find a method for eliminating these microorganisms from the cell cultures.

Methods employed: a) Treatment of cultures with massive, subtoxic doses of various tetracylines.
B) Thermal inactivation of L-forms. Hep II cells exposed to 40°C to 41°C for various intervals in order to inactivate the microorganism.

Major findings: a) None of the antibiotics employed caused a permanent cure of the cell cultures. The infection recurred after withdrawal of the drug. b) High temperature incubation of cell culture failed to eliminate the infection.

Serial No. DBS 23
1. Laboratory of Viral Products
2. Tissue Culture Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Biological Properties of Ribonucleic Acid From Virulent and Attenuated Poliovirus.

Principal Investigator: Dr. Paul Gerber

Other Investigator: Dr. Ruth Kirschstein

Cooperating Units: None

Man Years
Total: 1.0
Professional: 0.6
Other: 0.4

Objectives: To study the relationship of neurovirulence of Type I poliovirus to viral ribonucleic acid (RNA)

Methods employed: Infective RNA extracted from virulent and attenuated polioviruses was injected intraspinally into mice and was also tested for *in vitro* markers of neurovirulence.

Major findings: The results suggest that the infective RNA from Type I poliovirus carries the genetic information which determines the degree of neurovirulence.

Significance to Division: Basic information on virus-host interaction.

Proposed course: This phase of this study is completed. A paper has been submitted for publication.

Part B included Yes /X/ No /__/

Serial No. DBS-23

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications

Paul Gerger and Ruth Kirschstein, Biological Properties of Ribonucleic Acid From Virulent and Attenaated Poliovirus.

Submitted to J. Exp. Med.

Serial No. DBS-24

1. Laboratory of Viral Products
2. Section on Testing
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Functions Associated with the Poliomyelitis Vaccine Safety Testing Program

Principal Investigator: Dr. Samuel Baron

Other Investigators:
Dr. Ruth Kirschstein
Dr. George A. Hottle
Dr. Eugene V. Barnett
Dr. B. William Uhlendorf
Dr. C. W. Hiatt
Dr. Gerald L. Van Hoosier

Cooperating Units: None

Man Years:

Total: 3	Patient Material:
Professional: 1	Outside NIH
Other: 2	

Project Description:

Sub-project A: Concentration of Poliomyelitis Vaccine and the Sensitivity of the Monkey Safety Test.

Objectives: To increase the sensitivity of the monkey safety test by increasing the effective volume tested in monkeys.

Methods Employed: Studies were continued in which experimentally infected poliomyelitis vaccines were concentrated 100-fold by the ultracentrifuge-gelatin method developed in this laboratory. The concentrate is inoculated intraspinally in one group of monkeys and unconcentrated material was inoculated into an equivalent group of monkeys. Comparison of paralytic rates, pathological findings and virus isolationswas made between the two groups.

Major Findings: Monkeys inoculated with concentrated experimental vaccines were infected with poliovirus more frequently than those monkeys inoculated with equivalent but unconcentrated material. The increased infectivity was correlated with the degree of concentration. This monkey safety test using concentrated vaccine was more sensitive or at least equally sensitive to poliovirus in experimental vaccines than was the monkey kidney cell culture test when equal volumes of vaccine were used.

Significance to the Division: The use of concentrated vaccine in the monkey safety test appears to be the most sensitive technique developed for the detection of residual live virus in poliomyelitis vaccine.

Proposed Course: To write the results for publication and inclusion into the regulations for testing poliomyelitis vaccine.

Subproject B: Sensitivity of Cell Cultures to Poliovirus

Objectives: To control sensitivity of cell cultures used for the poliomyelitis vaccine tissue culture safety test.

Methods Employed: Poliovirus is titrated in each cell lot both before and after use in the vaccine test. Only cell lots with maximum sensitivity are approved for use in the test. Serums, which may be used in the cell culture system, are tested for poliovirus inhibitors. Only inhibitor-free serums are approved.

Major Findings: The great majority of cell cultures have been determined to be highly sensitive to poliovirus and therefore suitable for the safety test. Also some calf serum lots have been selected for use after demonstration of lack of poliovirus inhibitor.

Significance to the Division: Provides control of sensitivity of poliomyelitis vaccine cell culture test.

Proposed Course: This control activity to continue.

Subproject C: Isolation and Virulence Characterization of Attenuated Live Poliovirus from Stools of Vaccine Recipients and from Monkey Tissues

Objectives: To evaluate virulence and capacity to revert to virulence of live poliovirus vaccine strains.

Methods Employed: Isolation of virus from stool or tissues followed by characterization of virulence by in vitro genetic markers and in vivo monkey virulence.

Major Findings: The relative virulence of various proposed vaccine strains has been determined. Also some of these strains were shown to revert towards virulence by in vitro markers after passage through the human intestinal tract.

Significance to the Division: Provides information relative to selection of suitable strains for live poliovirus vaccine.

Proposed Course: Continue evaluation of reversion to virulence by poliovirus in human stool specimens. Correlate virulence in man with virulence in monkeys and by virulence as determined *in vitro* markers.

Subproject D: Study of poliovirus inhibitor in the central nervous system of monkeys.

Objectives: To characterize and determine methods for eliminating an interfering poliovirus inhibitor which was discovered in the nervous system of rhesus monkeys and to evaluate its importance in disease.

Methods Employed: Suspension of nervous tissue is mixed with poliovirus and the mixture assayed for virus by the plaque test.

Major Findings: The poliovirus inhibitor is unlike antibody, does not irreversibly inactivate virus and does not inhibit some Coxsackie and ECHO viruses.

Significance to the Division. Will provide information to determine methods for elimination of inhibitor which interferes with poliovirus isolation. Also will provide information relative to importance of inhibitor in pathogenesis of poliovirus infection of the nervous system.

Proposed Course: Determination of (1) chemical treatment which will inactivate inhibitor (2) mechanisms of action of inhibitor and (3) effect of inhibitor on experimental poliovirus infections of animals.

Subproject E: Correlation of Antibody Response to Poliomyelitis Vaccine in Man, Monkey, and Chick.

Objectives: To provide information so that animal potency requirements for poliovaccine may be established at a level which will provide a desired response in man.

Methods Employed: Varying amounts of poliovirus vaccine antigen were administered to children and their serums assayed for poliovirus antibodies at suitable intervals. Animal potency tests were performed on the same vaccines.

Patient Material: In cooperation with the District of Columbia Department of Health, a group of non-vaccinated children were recruited for the study.

Major Findings: Data is being analyzed.

Significance to the Division: Improvement of potency standards for poliomyelitis vaccine.

Proposed Course: Analysis of data and proposal of suitable animal potency requirements.

Subproject F: Development of a Semiautomatic Diluting Machine

Objectives: To develop a machine which will perform most of the time-consuming dilutions which are required in an immunology laboratory.

Methods Employed: A design of such a machine was discussed with the instrument shop at NIH. Following completion of the machine tests were performed to evaluate its performance.

Major Findings: The machine was found capable of accurately diluting antibody, hemagglutination or dye simultaneously in 12 parallel operations. A major time savings was observed.

Significance to the Division: A machine is now in use which saves a great deal of time and money and which enhances the accuracy of diluting operations.

Proposed Course: Description of the machine in a paper to be published in the scientific literature.

Part B included Yes/ X / No/____/

Serial No. DBS 24

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications:

Murray, R., Kirschstein, R., Van Hoosier, Jr., G., Baron S., Comparative Virulence for Rhesus Monkeys of Poliovirus Strains Used for Oral Administration, 1st International WHO Conf. Live Poliovirus Vaccines, Scientific Publication, No. 44, pg. 39, 1959.

Serial No. DBS 24a
1. Laboratory of Bacterial Products
2. Hemophilus Studies
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Immunity to Poliovirus and Other Virus Infections

Principal Investigator: Dr. Samuel Baron

Other Investigators:
Dr. Robert Friedman
Dr. Eugene V. Barnett
Dr. Wilfred R. Ehrmantrout, Medical Director, D. C. Children's Center, Laurel, Maryland
Dr. Robert S. Goldsmith, Laguna Honda Home, San Francisco, California
Dr. Sam Silbergeld
Dr. James E. Boyland, D. C. Children's Center Laurel, Maryland
Dr. John P. Nasou, NIAID
Dr. George M. Owen, NIAID

Man Years:
Total: 2
Professional: 1/2
Other: 1 1/2

Patient Days: Outside NIH

Subproject A: Rapid Immunization with Poliomyelitis Vaccine

Objectives: To determine a dosage schedule of poliovaccine which will result in early appearance of immunity and therefore be of value during epidemics of poliomyelitis.

Methods Employed: Various dosage schedules were employed in children and adults and serial bleedings were assayed for neutralizing antibody to poliovirus.

Patient Material: Children at the Children's Center, and in the District of Columbia through the D. C. Department of Public Health.

Major Findings: A large single inoculation (10 ml) of average or high potency poliovaccine was capable of evoking antibody within 1 to 2 weeks of inoculation.

Significance to the Division: Method to provide protection for non-immune persons during a poliomyelitis epidemic.

Proposed Course: Determination of dosage schedule which will provide rapid immunization, maintain antibody throughout the immunization period and produce high levels of antibody.

Subproject B: Effect of Administration of Human Gamma Globulin on Infectious Diseases of Children and Aged Persons.

Objectives: Determine the ability of gamma globulin to reduce the incidence and severity of certain common infectious diseases.

Methods Employed: This double-blind study in 381 institutionalized elderly adults and 115 children utilized 3 or 6 monthly 1 m. inoculations of 0.15 or 0.4 ml. GG per pound. The two populations were each randomly divided into three approximately equal groups, viz. GG, saline placebo, and non-inoculated recipients.

Patient Material: Institutionalized children and elderly adults at D. C. Children's Center and Laguna Honda Home.

Major Findings: Infections among all adults occurred in 3 main categories, totaling 261 episodes and 422 days; among children 7 main categories, totaling 986 episodes and 3588 days. Significant prophylaxis, expressed as % protection based on episodes of illness (P_e) and % protection based on days (P_d), was observed among children for mumps ($P_e = 74$, $P_d = 69$), whereas no significant prophylaxis was noted for upper respiratory infections, pneumonia and bronchopneumonia, and fever of unknown origin.

Significance to the Division: This investigation suggests some new limitations and values of GG as a prophylactic agent. Also the information may lead to successful prophylaxis of mumps in man.

Proposed Course: To determine effectiveness of gamma globulin of known antibody content given after exposure to mumps virus.

Subproject C: Antibody to Enteroviruses in the Serums of Persons with Hypogammaglobulinemia.

Objectives: To determine whether newly developed, highly sensitive techniques for detection of viral antibody could demonstrate antibody in the serums of persons with hypogammaglobulinemia.

Methods Employed: Immunoinactivation test for virus antibody in serum.

Patient Material: Serums from outpatients and inpatients of LID of NIAID.

Major Findings: Detection of extremely low levels of antibody to enterovirus in these serums. The distribution of antibody was normal but diminished in quantity. In one case, low levels of antibody to poliovirus were detected following immunization with poliovaccine.

Significance to the Division: Information which leads to changed concept of the disease and may explain why these persons are normally resistant to viral infections.

Proposed Course: Fractionation of serum proteins to determine location of these low level antibodies.

Subproject D: Effect of hypersensitivity on immunity to virus infections.

Objectives: To determine whether a state of hypersensitivity to viral antigens is of importance to resistance to virus infection in experimental animals.

Methods Employed: Animals have been made delayed hypersensitive to viral antigens by various methods of immunization. Skin testing and challenge are being carried out on these animals.

Major Findings: Delayed hypersensitive states have been established to vaccinia and polioviruses following inoculation of live or dead materials.

Significance to the Division: Studying a possible mechanism for animal resistance to virus infection.

Proposed Course: Further investigation of the relationship of delayed hypersensitive states to antibody response to viral agents and testing of resistance of hypersensitive animals to virus infections. Establishment of whether delayed hypersensitivity is a necessary precursor to antibody production against viral antigens and establishing what, if any, resistance is conferred upon animals which are made delayed, hypersensitive in the absence of antibodies.

Subproject E: Distribution of antibody in Serum Proteins During Immunization and Diseases Affecting Gamma Globulin

Objectives: To fractionate serum in order to locate and identify antibodies in the various fractions of sera obtains from patients in unusual immunologic states.

Methods Employed: Cellulose resin columns - DEAE and CM to fractionate serum and immunoinactivation for evaluation of serum antibody content.

Patient Materials: Sera from volunteers from Employee Health Service, NIH, who received 19 cc. of polio vaccine and from hypogammaglobulinemic patients of the NIAID.

Major Findings: Methods have been applied by which serum may easily be divided into various pure fractions in our laboratory.

Significance to the Division: Better understanding of what factors in serum are responsible for resistance to virus infection and of immune process in general.

Proposed Course of Project: Fractionation of EHS and hypogammaglobulinemic sera together with any sera we can obtain from patients with disorders in the RE system, i.e., lymphomas, leukemias, myeloma, cirrhosis, etc.

Part B included Yes ☒ No ☐

Serial No. [illegible]

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications:

Baron, S., Barnett, E. V., Burch, B. L., Lynch, J. M., and Ehrmantraut, W. R., Rapid Immunization with Poliomyelitis Vaccine. New England Journal of Med., 260, 966-969, 1959.

Driscoll, E. J., Ship, I. I., Baron, S., Stanley, H. R., Jr., Utz, J. P., Chronic Aphthous Stomatitis, Herpes Labialis and Related Conditions: Combined Clinical Staff Meeting of the National Institutes of Health. Annals of Internal Medicine, 50, 1475-1496, 1959.

[illegible]
1. [illegible]
2. Section on [illegible]
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Experimental Studies with Viruses and Cell Cultures.

Principal Investigator: Dr. Samuel Baron

Other Investigators: Dr. Eugene V. Barnett
Dr. C. W. Hiatt

Man Years:
Total: 2
Professional: 1/2
Other: 1 1/2

Patient Days: None

Subproject A: Comparative Study of Ultraviolet Light Inactivation of Animal Viruses.

Objectives: Determination of the ultraviolet light inactivation properties of a number of animal viruses for characterization and classification.

Methods Employed: Aqueous suspensions of various viruses are exposed to ultraviolet radiation. Samples are taken at appropriate intervals and assayed for infectivity. Plots are then made for each virus relating decline of infectivity with time of irradiation. Intensity of irradiation is measured with an Archer radiation meter and also by inactivation of a standard virus preparation (poliovirus Type III, Saukett strain).

Major Findings: Inactivation rate and slope are correlated with relationship of viruses as determined by other properties. Inactivation rate is not proportionate to virus size as is true of bacteriophage.

Significance to the Division: Basic information on the physical property of ultraviolet light inactivation of viruses. Potential application for identification and classification of viruses.

Proposed Course: Write paper for publication.

Subproject B: Plaque Size Inhibition as an Assay of Antibody and Inhibitor to Poliovirus.

Objectives: Development of a new and easier technique for the assay of poliovirus antibody by the use of a method which does not require serial dilution of serum.

Methods Employed: Monolayers of monkey kidney cell culture are infected with small amounts of poliovirus and then overlayed with agar containing immune serum. Diameters of resulting plaques are measured in comparison to serum-free control plaques. Results are analysed for correlation with known serum antibody titer, reproducibility and variation among serum from different species.

Major Findings: Plaque size inhibition is well correlated with poliovirus antibody titers as measured by accepted tests. Reproducibility appears somewhat better than the metabolic inhibition test. Antiserum from man, chicken, and monkey are indistinguishable by the plaque size inhibition test. In tests of over 300 sera, methods have been developed for rapid large scale application.

Significance to the Division: Potential application in the serology unit for potency tests of poliomyelitis vaccine in animals and man. Theoretical implications relating to pathogenesis of poliomyelitis.

Proposed Course of Project: Write for publication.

Subproject C: Antigenic Crossing of the 3 types of poliovirus.

Objectives: To determine the antigenic relationships among the 3 types of poliovirus.

Methods Employed: Serums from rabbits immunized with plaque-purified poliovirus assayed for antibody by extremely sensitive techniques (Immunoinactivation).

Major Findings: Rabbits immunized to a single poliovirus Type I respond with high levels of antibody to the homotypic type and with extremely low levels of antibody to 1 or 2 heterotypic types.

Significance to the Division: Basic information on the sharing of common antigens by polioviruses.

Proposed Course: Determine the characteristics of the low level antibody response.

Subproject D: Viral Sensitivity of Cell Culture Maintained in Skim Milk Medium.

Objectives: To determine suitability of skim milk medium for viral assays in cell cultures.

Methods Employed: Comparative assay of 22 viruses in cell cultures maintained with skim milk medium and with an established maintenance medium.

Objectives: Development of a new and easier technique for the assay of poliovirus antibody by the use of a method which does not require serial dilution of serum.

Methods Employed: Monolayers of monkey kidney cell culture are infected with small amounts of poliovirus and then overlayed with agar containing immune serum. Diameters of resulting plaques are measured in comparison to serum-free control plaques. Results are analyzed for correlation with known serum antibody titer, reproducibility and variation among serum from different species.

Major Findings: Plaque size inhibition is well correlated with poliovirus antibody titers as measured by accepted tests. Reproducibility appears somewhat better than the metabolic inhibition test. Antiserum from man, chicken, and monkey are indistinguishable by the plaque size inhibition test. In tests of over 300 sera, methods have been developed for rapid large scale application.

Significance to the Division: Potential application in the serology unit for potency tests of poliomyelitis vaccine in animals and man. Theoretical implications relating to pathogenesis of poliomyelitis.

Proposed Course of Project: Write for publication.

Subproject C: Antigenic Crossing of the 3 types of poliovirus.

Objectives: To determine the antigenic relationships among the 3 types of poliovirus.

Methods Employed: Serums from rabbits immunized with plaque-purified poliovirus assayed for antibody by extremely sensitive techniques (Immunoinactivation).

Major Findings: Rabbits immunized to a single poliovirus Type I respond with high levels of antibody to the homotypic type and with extremely low levels of antibody to 1 or 2 heterotypic types.

Significance to the Division: Basic information on the sharing of common antigens by polioviruses.

Proposed Course: Determine the characteristics of the low level antibody response.

Subproject D: Viral Sensitivity of Cell Culture Maintained in Skim Milk Medium.

Objectives: To determine suitability of skim milk medium for viral assays in cell cultures.

Methods Employed: Comparative assay of 22 viruses in cell cultures maintained with skim milk medium and with an established maintenance medium.

Major Findings: All viruses, except adenoviruses manifest full titer in cell cultures maintained with skim milk medium. Due to a cell-mediated effect adenovirus titers are one $\log_{10}$ lower in skim milk medium than in Ginsberg's medium.

Significance to the Division: Confirms the suitability of skim milk medium as a maintenance medium.

Proposed Course: Subproject completed.

Part B included Yes /X/ No / /

Serial No. DBS- 35

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications:

Gochenour, A. M. and Baron, S., Viral Sensitivity of Cell Cultures Maintained with Skim Milk Medium. Proc. Soc. Exp. Biol. & Med., 102, 230-233, 1959.

Barnett, E. V., and Baron, S., Cell Culture Produced Plasminogen Activator, Proc. Soc. Exp. Biol. and Med., 102, 308, 1959.

Serial No. DBS-26
1. Laboratory of Viral Products
2. Section on Testing, Serology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Antigenic Potency of Poliomyelitis Vaccine as Determined in Chicks and in Monkeys.

Principal Investigator: Dr. B. Wm. Uhlendorf

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 6.5
Professional: 0.5
Other: 6.0

Patient Days: None

Project Description:

Objectives: 1. To determine the antigenicity of poliomyelitis vaccine.

2. Determine the relative merits of the two tests and the minimum potency requirement, as determined by the chick test, of domestic poliomyelitis vaccines.

Methods: The methods are those outlined in the PHS regulations and in the scientific literature.

Major Findings: 1. During the year 55 vaccines were tested by the chick test involving approximately 8000 chicks.

2. During the year 47 vaccines were tested by the monkey test involving approximately 500 monkeys.

3. From theoretical, practical and statistical evaluations, the chick test is believed to be the more valid test.

Significance to the Program of DBS: The comparison of the monkey-test with the chick-test is necessary to establish minimum requirements for poliomyelitis vaccine in the probable event that the chick test will replace the monkey test in the regulations as the method of estimating antigenic potency.

Part B included Yes ☐ No ☒

Serial No. [illegible]
1. Laboratory of [illegible]
2. Section on [illegible]
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: The Measurement of Antipoliovirus Antibodies By the Metabolic Inhibition Test (MIT)

Principal Investigator: Dr. B. Wm. Uhlendorf

Other Investigators: Mrs. Beatrice Burch

Cooperating Units: The tissue culture unit supplies suspensions of monkey kidney cells.

Man Years:
Total: 3
Professional: 1/2
Other: 2 1/2

Project Description:

Objectives: 1. To screen for the presence of anti-poliovirus antibodies in pre-vaccination sera of monkeys used for the monkey potency-test, in sera from monkeys used for the safety test, and in sera from chicks used for the chick potency test. To determine the relative antibody content of post-vaccination sera from potency-test monkeys. To determine the anti-poliovirus antibody titer of human sera collected in field studies, and of commercial gamma-globulin preparations for standardization purposes.

2. To examine the effect of variation of technique and of agents used for the MIT on the reliability of the test.

Methods: The methods are those outlined in the scientific [illegible]

Major Findings: (1) during the year approximately 2700 sera of all kinds were titered for antibody content, and 12000 sera screened.

(2) by defining and approximately adjusting the [illegible] techniques it has been possible to improve to a considerable [illegible] the reliability of the MIT.

Significance to the Program of DBS: Integral part of DBS [illegible] program.

Part B included Yes ☐ No ☒

Serial No. DBS-4
1. Laboratory of [illegible]
2. Section on Monkey Testing
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Monkey Safety Test for Poliomyelitis Vaccine
and Adenovirus Vaccine.

Principal Investigator: Dr. Ruth Kirschstein

Other Investigators: Dr. Gerald Van Hoosier
Dr. Gerald Borman

Cooperating Units: None

Man Years:
Total: 12.0
Professional: 1.0
Other: 11.0

Project Description:

Objectives: To test poliomyelitis and adenovirus vaccine for the presence of residual active virus particles which can be detected by the direct inoculation of monkeys.

Methods Employed. Outlined by the PHS Regulations for the above vaccines.

Major Findings: During 1959, 56 of 188 poliomyelitis vaccines one of 15 adenovirus vaccines were tested for safety in monkeys. 1140 monkeys were examined. Histologic lesions were found in 7 monkeys. These were not lesions of poliomyelitis and no virus was recovered from the tissues of these monkeys

Significance to the Program of DBS: Integral part of the DBS program.

Proposed Course: This endeavor will continue in 1960 on the same scale.

Part B included Yes /__/ No /X/

Serial No. DBS-19
1. Laboratory of Viral Products
2. Section on Monkey Testing
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the Neurovirulence of the Attenuated Poliovirus (live vaccine) Strains in Monkeys.

Principal Investigator: Dr. Ruth L. Kirschstein

Other Investigators: Dr. Samuel Baron
Dr. Gerald Van Hoosier
Dr. Gerald Borman
Mr. George Rusten

Cooperating Units: None

Man Years:
Total: 2
Professional: 1
Other: 1

Project Description:

Objectives: To compare the monkey neurovirulence of the nine strains of attenuated polioviruses that have been used in field trials. This comparison was essential in order to develop recommendations relating to the manufacture of live poliovirus vaccine.

Methods: Comparative titrations in the strains by intraspinal and intrathalamic inoculation into monkeys were carried out. A total of 545 monkeys were used in this study. Careful histopathologic study of the spinal cord and brain tissue was carried out.

A study of the virulence of these strains when inoculated peripherally into monkeys is not underway.

Major Findings: All the strains are capable of producing lesions of poliomyelitis when inoculated intraspinally. Some of the strains, however, are completely attenuated when inoculated intrathalamically.

Significance to the Program of DBS: This study has provided an important basis for the recommendations relating to the manufacture of live poliovirus vaccine.

Proposed Course: Safety testing of vaccines submitted by manufacturers will be carried out in accordance with methods developed in this study. Further exploration of the relationship of monkey neurovirulence to human neurovirulence is planned.

Part B included Yes ☒ No ☐

Serial No. DBS 29

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Murray, R., Kirschstein, R. L., Van Hoosier, G. and Baron, S.: Comparative Virulence for Rhesus Monkeys of Poliovirus, WHO Conference on Live Poliovirus Vaccines, June 1959.

Serial No DBS-30
1. Laboratory of Viral Products
2. Testing Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: The Pathogenesis of Viral Diseases.

Principal Investigator: Dr. Ruth L. Kirschstein

Other Investigators: Dr. Lawrence Kilham
Dr. Alan Rabson, NCI
Dr. C. P. Li
Dr. Bernice Eddy
Dr. Paul Gerber
Dr. Gerald Van Hoosier
Dr. Gerald Borman

Cooperating Units: Laboratory of Bacterial Products,
Pathologic Anatomy Branch, NCI.

Man Years:
Total: 0.5
Professional: 0.5
Other: None

Project Description:

Objectives: To study the pathogenesis of the various viral entities that other members of the Division are studying.

Methods: Gross autopsies and histological examinations were carried out on infected animals.

Major Findings: 1. (With Drs. Kilham and Rabson) Fibroma viruses of squirrels and rabbits cause widespread disease when inoculated into suckling animals. The squirrel fibroma virus causes lesions in the lungs of suckling squirrels which resemble pulmonary adenomatosis in man and other animals. This has now been found in a total of six such animals. Suckling rabbits develop generalized fibroma tumors but the lesions of pulmonary adenomatosis are not seen.

2. (With Dr. Li) Sarcoma 180 has been sucessfully transplanted from mice to hamsters by intracerebral inoculation. The polyoma virus has been isolated from Sarcoma 180 in mice and its presence confired by the production of characteristic tumors in hamsters. The relationship of the virus to Sarcoma 180 is in question.

3. (With Dr. Rabson) The pathogenesis of the intracranial sarcomas produced in hamsters by the polyoma virus has been studied in detail.

4. (With Drs. Eddy and Borman) Polyoma virus induces the production of subcutaneous nodules resembling "fibromatoses" in suckling rabbits. Subcutaneous nodules produced in guinea pigs are also being studied.

5. (With Dr. Gerber) The infectious RNA from mouse-adapted poliovirus has been found to produce specific lesions of poliomyelitis when inoculated intraspinally in mice.

6. (With Drs. Van Hoosier and Borman) Continued study of the agent that produces fibroma-like lesions in monkeys is underway. Attempts are being made to adapt the agent to another animal host.

Part B included Yes / X / No / /

Serial No. DBS-30

Part B: Honors, Awards and Publications

Publications:

Kirschstein, R. L., Rabson, A. S., and Kilham, L. Pulmonary Lesions Produced by Fibroma Viruses in Squirrels and Rabbits, Cancer Research, 18: 1340-1344, (1958)

Eddy, B. E., Stewart, S. E., Kirschstein, R. L., and Young, R. D. Inductive of Subcutaneous Nodules in Rabbits with the S. E. Polyoma Virus, Nature, 183: 766, (1959)

Li, C. P., Kirschstein, R. L., and Jahnes, W. G. Intracerebral Passage of Sarcoma 180 in Mice and Hamsters, Proc. Soc. Exp. Biol. and Med., 101; 252, (195 ,

Rabson, A. S. and Kirschstein, R. L. Intracranial Sarcomas Produced by Polyoma Virus in Syrian Hamsters, Arch. Path. (in press)

Serial No. DBS-[illegible]
1. Laboratory of Viral Products
2. Research Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the Inactivation of Viruses.

Principal Investigators: Dr. C. W. Hiatt
Dr. Jerome J. Helprin

Other Investigators: Mrs. Eleanor Kaufman
Miss Dorothy Moore
Mr. William G. Jahnes

Cooperating Units: None

Man Years:
Total: 2.75
Professional: 1.75
Other: 1.00

Patient Days: None

Project Description:

Objectives: (1) To gain information about the fundamental nature of the various biological properties of viruses through the study of the mechanisms of inactivation of these properties by chemical and physical agents.

(2) To develop an understanding of processes of inactivation which are applicable to the preparation of viral vaccines.

Methods Employed: Various animal and bacterial viruses are cultivated in cell cultures and the infective fluids from these cultures are subjected to treatment with graded doses of the inactivating agent. Samples taken at the different dose levels are assayed for infectivity, and the kinetic relationship between dose and survival ratio is established. From the characteristics of the survival curve inferences are drawn about the properties of the virus and the mechanisms of the inactivating process.

Respirometric methods are used to investigate the mode of action of the virus upon its host cell and to search for variations which may result from treatment of the virus with inactivating agents.

Major Findings: (1) Relative susceptibility of animal viruses to inactivation by the photodynamic action of toluidine blue.

Extending our earlier investigation of this subject, we have determined approximate velocity constants for the inactivation of infectivity of several additional viruses by visible light in the presence of 0.6 mg% of toluidine blue. The viruses studied to date may be classified as follows:

Susceptible	Resistant
T2r+ coliphage	poliomyelitis I, II, and III
T3 coliphage	ECHO-1
Eastern equine encephalomyelitis	Coxsackie A-9 and B-2
vaccinia	
adenoviruses 3, 4 and 7	
simian virus 1	
rabies	
B-virus	
ECHO-10	
measles	
fibroma	
myxoma	
SE polyoma	

(2) Differential inactivation of B-virus in suspensions of live poliovirus.

Photodynamic treatment of mixtures of B-virus and poliomyeli virus destroys all B-virus infectivity (within the limits of sensitivity of the test system) but does not significantly reduce the infectivity of the poliovirus. This process appears to be of practical importance as a possible step in the preparation of live poliovirus vaccines.

(3) Development of continuous-flow irradiation equipment.

A water-jacketed glass helix surrounding a cylindrical incandescent light source has been employed for continuous-flow irradiation of fluids with visible light. This apparatus has been calibrated using suspensions of T3 coliphage sensitized with toluidine blue. With a 40-watt light source, phage suspensions containing 10^5 P.F.U./ml. may be sterilized at a flow rate of 12 ml./min. A 1000-watt light source under construction is expected to permit flow rates to be increased to at least 300 ml./min.

At the higher flow rate, this apparatus will be suitable for pilot-plant or small scale production applications.

(4) Mechanism of inactivation of viruses by photodynamic action.

T2 coliphage, treated with toluidine blue and irradiated with visible light, can infect host cells in a manner similar to infection by inactivated phage, inhibiting cell growth but not cell respiration. The inactivation of this virus by photodynamic action must therefore affect an event which occurs subsequent to infection of the host, possibly interfering with intracellular multiplication of the phage. These results extend the earlier finding that photodynamically inactivated phage can still attach to the cell and induce "lysis from without."

(5) Photodynamic action of proflavine on bacteriophage.

T2r+ coliphage becomes progressively sensitive to visible light when treated with 1×10^{-5} M proflavine (diaminoacridinium monohydrogen sulfate). Photodynamic inactivation of the sensitized virus proceeds at a rate which depends upon the time of contact between virus and dye. The survival ratio N/N_o of the phage at constant light intensity (I) may be expressed as:

$$\frac{N}{N_o} = e^{-I(at + \frac{bt^2}{2})}$$

in which *t* is the time of exposure to light. The constant *a* in this equation is a measure of the sensitivity of the phage at the beginning of irradiation; *b* is the rate at which further sensitization occurs during the irradiation.

a was determined for phage suspensions which had been treated with proflavine for various periods of time in the dark at 10, 25, and 37°C. The value of this constant appears to be an index of the extent of dye binding at critical sites in the phage particle. If *a* is assumed to be directly proportional to the amount of dye taken up, then the energy of activation for the dye uptake reaction is calculated to be 10.6 kcal/mole.

(6) Influence of temperature on the rate of uptake of toluidine blue by T2 coliphage.

The Arrhenius energy of activation for specific binding of toluidine blue by T2r+ coliphage at critical sites was found to be 28.3 kcal/mole, which is of the same order of magnitude as the energy of activation for inactivation of this phage with formaldehyde.

(7) Photodynamic action of toluidine blue on ECHO-10 virus.

ECHO-10 virus is decidedly different from other viruses of enteric origin which we have tested in that it is relatively susceptible to inactivation by the photodynamic action of toluidine blue. Uptake of dye by this virus is very slow, however, and there are some features of the dye uptake reaction which resemble the corresponding reaction with T2 coliphage. This finding leads to the interesting speculation that ECHO-10 virus has a demonstrable membrane with selective permeability properties.

(8) Preliminary experiments with the sodium pentachlorophenate actinometer.

Dilute solutions of sodium pentachlorophenate (NaPCP) are altered by exposure to ultraviolet light of 2537 A. The alteration may be detected by observation of the ultraviolet absorption spectrum or by measuring the capacity of the solution to promote the solubility of methylene blue in chloroform. A first-order decay curve is followed by the reaction, and the velocity constant is proportional to light intensity. The system promises to be useful as a means of measuring actual light intensity in thin-film irradiation equipment.

Significance to the Program of DBS: The continued investigation of the mode of action of virus inactivants is expected to contribute to the fundamental basis for the control of viral vaccine

Proposed Course of Project: This project will be continued.

Part B included Yes /X/ No / /

Serial No. DBS-31

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publication other than abstracts from this project:

Helprin, J. J., and Hiatt, C. W. Photosensitization of T2 Coliphage with Toluidine Blue. J. Bacteriol., 77:502-505, 1959.

Kaufman, E., and Hiatt, C. W. Photodynamic Action of Proflavine on T2 Coliphage. Virology, 9:478-479, 1959.

Hiatt, C. W., Kaufman, E., Helprin, J. J. and Baron, S. Inactivation of Viruses by the Photodynamic Action of Toluidine Blue. J. Immun., (In press).

Serial No. DBS-32
1. Laboratory of Viral Products
2. Research Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Physical and Chemical Characterization of viruses.

Principal Investigator: Dr. C. W. Hiatt

Other Investigators: Mr. John F. Thiel

Cooperating Units: None

Man Years:
Total: 0.50
Professional: 0.50
Other: None

Patient Days: None

Project Description:

Objective: To obtain information about the physical dimensions and chemical composition of animal viruses and to correlate this information with biological properties.

Methods Employed: Standard methods of physical and chemical analysis, including ultracentrifugation and electron microscopy, are supplemented by specialized techniques for concentrating and purifying viruses.

Major Findings: None. Activity on this project has consisted entirely in the training of an electron microscopist.

Significance to the Program of DBS: The development of methods for the rapid characterization of newly identified viruses will assist in the testing of viral vaccines for safety and purity.

Proposed Course of the Project: This project will be continued. Active investigation of the subject will be resumed upon completion of the present program of training.

Part B included Yes /___/ No /_X_/

Serial No. [illegible]
1. Laboratory of Viral Products
2. Research Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Effect of Sunlight on the Molluscicidal Activity of Sodium Pentachlorophenate Solutions.

Principal Investigator: Dr. C. W. Hiatt

Other Investigators: Dr. Louis J. Olivier
Dr. Willard T. Haskins

Cooperating Units: Laboratory of Tropical Diseases, NIAID

Man Years:
Total: 0.30
Professional: 0.20
Other: 0.10

Patient Days: None

Project Description:

Objectives: To determine the effect of sunlight as a variable affecting the efficacy of sodium pentachlorophenate as a molluscicide in ponds and streams.

Methods Employed: Aqueous solutios of sodium pentachlorophenate (NaPCP) are irradiated with light of known wavelength and intensity for graded periods of time. These solutions are then assayed by both chemical and biological methods to determine residual NaPCP.

Major Findings: NaPCP is altered by exposure to ultraviolet light in the wavelength region 2900 to 3300 Å, corresponding to a maximum at 2180 Å in the UV absorption spectrum for this compound. Molluscicidal activity and chemical identity are lost concomitantly. The concentration of intact NaPCP as a function of time of irradiation may be expressed as

$$C_o = Ce^{\frac{-kI\ t(1-e^{aL})}{aL}}$$

in which C and C_o are NaPCP concentrations at zero time and time t respectively, k is the velocity constant for the reaction, I_o is

the incident light intensity, a is the absorbance [illegible] and L is the depth of the body of liquid.

These findings serve to explain the unexpectedly [illegible] in certain areas of the world when NaPCP was used in [illegible] in an effort to control the snail vector of schistosomiasis. Recommendations for the use of NaPCP in the field will be [illegible] to allow for the effect of sunlight

Significance to the Program of DBS: This project is not directly related to the DBS research program. It was undertaken as a cooperative venture with NIAID because the methods used in this investigation were of general interest and were closely allied to those used in other investigations in progress.

Proposed Course of the Project: This project will be terminated upon completion of a manuscript describing the results.

(Part B not included)

Serial No. DBS-34
1. Laboratory of Viral Products
2. Research Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: **Development of Methods for the Chemical Analysis of Biological Products.**

Principal Investigator: **Dr. C. W. Hiatt**

Other Investigators: **Mr. Robert Blereau**

Cooperating Units: None

Man Years:
Total: 0.30
Professional: 0.30
Other: None

Patient Days: None

Project Description:

Objective: To develop specialized methods of chemical analysis which are applicable to various biological products and which pertain to safety, purity, potency, efficacy or stability of the product.

Methods Employed: Standard methods of chemical analysis are modified and new methods of analysis are devised as necessary.

Major Findings: Two methods have been developed for the assay of total protein in viral vaccines of tissue culture origin.

1. The proteins in 10-ml sample are precipitated with tungstic acid and collected on a Millipore MP filter. The filter is washed with water according to a standard procedure, then with acetone, and is dried under suction. The filter disc is then rolled up, placed in a test tube, and leached with Na_2CO_3 solution to redissolve the protein. This solution is then treated with Folin-Ciocalteu reagent, and the protein is determined in the spectrophotometer by comparison with a standard curve. Protein concentrations in the range 0.01 to 0.10 mg% could be measured accurately by this method.

2. An alternate method, which is better adapted for the screening of large numbers of samples, was devised, using the insolubility of proteins in sulfosalicylic acid solutions as a basis for the measurement. Addition of a standard amount of

sulfosalicylic acid to a 10-ml sample of vaccine resulted in a stable turbidity which could be measured in a sensitive nephelometer. Protein concentration was estimated by comparison with a calibration curve. This method was found to be satisfactory for determination of protein concentrations as low as 0.005 mg%.

Significance to the Program of DBS: Variations in processes for the manufacture of poliomyelitis vaccine have made it essential to know the protein content of the vaccine in order to ascertain the safety and purity.

Proposed Course of the Project: This project will be continued.

Part B included Yes /_/ No /X/

Serial No. [illegible]
1. Laboratory of [illegible]
2. Research Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A:

Project Title: Properties of the Sera of Guniea Pigs Inoculated with Diphtheria Toxoid and Freund's Adjuvant.

Principal Investigator: Dr. C. W. Hiatt

Other Investigators: Dr. Sara E. Branham

Cooperating Units: None

Man Years:
Total: 0.10
Professional: 0.10
Other: None

Patient Days: None

Project Description:

Objectives: To determine a) the electrophoretic composition and b) the immunological character of diphtheria antitoxin produced in the guinea pig with the aid of Freund's adjuvant.

Methods Employed: Guinea pig antisera had been prepared as part of an earlier investigation by injecting diphtheria toxoid along with Freund's complete adjuvant containing *Mycobacterium butyricum*. The method of zone eldctrophoresis on paper strips was used to compare the protein composition of these sera with that of a pool of normal guinea-pig serum. A nephelometric method especially devised for the purpose was used to obtain precipitin curves.

Major Findings: There were significant absolute increases in the beta and gamma globulin fractions of these antisera. These alterations were regarded as a non-specific response to the stimulus of Freund's adjuvant.

Precipitin curves displayed only a zone of inhibition in antigen excess, indicating that the antitoxin in these guinea-pig sera was different in kind from that produced in the horse

Significance to the Program of DBS: Exploration of the possibility of using guinea-pig antisera for diphtheria flocculation tests may facilitate studies of the comparative immunogenicity of various

[illegible]

Proposed Course of the Project: This project will be terminated upon completion of a manuscript describing the results.

Part B included Yes ☐ No ☒

Serial No. DBS 30
1. Laboratory of Viral Products
2. Research Section
3. Bethesda, Md.

Projects Report
Calendar Year 1959

Part A.

Project title: Respiratory Viruses
Principal investigator: Dr. Bernice E. Eddy
Other investigators: None
Cooperating Units: None

Man Years:

Total 8
Professional: 1
Other: 7

The unit is concerned with four primary projects and one minor project. These are: Influenza viruses, adenoviruses, vaccines for multiple respiratory agents, tumor viruses and pneumococci.

Methods employed:

The methods employed for these projects consist of animal inoculation, propagation of virus in animals, embryonated eggs and tissue cultures from various tissues from different animals, propagation of bacteria in culture mediae, centrifugation, differential filtration and serological methods.

II. Project title: Influenza Viruses

Man years:

Total 1 5/6
Professional: 1/3
Other: 1 1/2

Objectives: The objectives of the project are to control and improve influenza virus vaccines and to improve methods for evaluating the safety, purity, and potency of the product.

The disease influenza was not a major problem in the U. S. during 1959 but interest in immunization with influenza virus vaccines has continued. From December 7th, 1958, to December 7th, 1959, 62 lots of influenza virus vaccine were considered for release as follows:

Vaccines	Lots submitted in latter part of 1958	Lots submitted in 1959	Lots released in 1959
Polyvalent military (six strains)	0	22	22
Polyvalent civilian (four strains)	6	37	40
Total	6	59	62

Major Work on the Project:

A. Potency tests on influenza virus vaccines. Potency tests for measuring the antigenic activity in mice were performed on 62 lots of vaccine submitted for release and on 2 lots of live virus vaccines from Russia.

B. Control of diagnostic influenza virus antigens and antisera. Only one diagnostic influenza virus antigen was submitted for consideration for license and none were submitted for release. No diagnostic antisera were submitted for release.

C. Hemagglutination as a measure of viral content of vaccines. The factors that influence this test continue to render investigation in an effort to simplify the test now being used.

Reference standards for influenza virus vaccines:

A. Two new reference influenza virus vaccines were obtained, tested, and distributed to the manufacturers. Both were adopted as official reference standards during 1959.

a. Vaccine Lot 911, was adopted as a reference for mouse antigenic tests for civilian formula influenza virus vaccines containing the PR8, Asian, Ann Arbor, and Great Lakes strains.

b. Vaccine Lot X2 was adopted as a reference for the mouse antigenic tests for military formula influenza virus vaccine containing the Swine, PR 8, Asian, Ann Arbor, Lee and Great Lakes strains.

c. Work has continued in an attempt to prepare a stable dried reference standard for the CCA test. A large pool of Jap 305 allantoic fluid has been prepared and dialyzed. Alliquot portions will be treated in different ways and tested for viral content in an effort to find a satisfactory method of preparation.

d. Studies on the antigenicity of current influenza virus strains. Because the antigenic composition of influenza virus strains that cause disease change every few years, a constant check on the strains currently causing disease is made and compared to the strains in the vaccines. During 1959, the PR301 strain was omitted from the civilian and military formula vaccines and the Ann Arbor strain, a more recent strain that differs antigenically from PR301, was substituted.

The general aim of the project is to fulfill the obligation of controlling and improving influenza virus vaccines. The proposed course of the project will follow along the same course as in 1959.

II. Project title: Adenoviruses

Man years: 2 1/6
Total: 1/6
Professional: 2

Objectives: The objectives of the project are to control and improve adenovirus vaccines and to improve methods for evaluating the safety, purity, and potency of the product.

Adenovaccines have been of considerable interest to the military since new troops are particularly susceptible to adenovirus infections. During 1959 another manufacturer was licensed to sell this product and still another manufacturer has submitted experimental lots for study. During the year, 14 lots were submitted for release and 14 lots were released.

Major work on the project:

A. Potency of adenovirus vaccines for 3, 4, 7. Work on different methods for determining the potency of adenovirus vaccines has been continued. One method involving the immunization of guinea pigs and the use of their sera in a serum-virus neutralization test in known numbers of HeLa cells was used to test all vaccines that have been submitted for release. Efforts are being continued to simplify and improve the test.

B. Studies of susceptibility of tissue culture cells for the adenoviruses and inhibitors for the viruses in normal sera. These studies are of importance in safety tests of adenovirus vaccines.

C. Reference vaccine. Monovalent vaccines for types 3, 4 and 7, were obtained and portions of each were pooled. The potency of the lot of vaccine was compared with the potency of a vaccine that had successfully been used in a field trial and which until recently was used as reference standard. The potency of type 7 was low and plans are underway to obtain a new monovalent vaccine for this type.

[illegible] Some studies are under way with these other types of [illegible] in anticipation of an adenovirus vaccine for children.

The general aim of the project is to fulfill the obligation of controlling and improving adenovirus vaccine. The proposed course of the project will follow along the same course as in 1959.

III. Project title: Vaccines for multiple respiratory agents.

Man years:

Total: 2 1/6
Professional: 1/6
Other: 2

Objectives: This project is for the purpose of controlling multiple antigen vaccines, particularly vaccines made from different viruses.

During 1959 a license was issued to one manufacturer for a vaccine that is made up of 4 strains of influenza virus and 3 strains of adenovirus, adsorbed on alum. Standards are already available for influenza and adenoviruses but the tests involve immunization of different species of animals by different routes.

Major work on the project: The multiple antigen vaccine that has been licensed involves the concentration of the adenovirus components. Adsorption on alum, and the discarding of much of the tissue culture fluid used in the propagation of the virus. This is different from the preparation of adenovirus vaccines. Extensive potency tests were carried out for both the adenovirus and influenza virus components and some work has been done in an effort to simplify the testing procedures. Nine lots of the combined vaccine were submitted and 5 lots were released.

The general aim of the project is to fulfill the obligation of controlling and improving the combined adeno and influenza virus vaccines and in anticipating the need to control other multiple antigen vaccines having to do with respiratory diseases.

IV. Project Title: Tumor Viruses (Most of this work has been done in conjunction with Dr. Sarah Stewart, NCI).

Man years: 1 1/2 1 5/6
Professional: 1/3
Other: 1 1/2

Objectives: The objectives of the project are to isolate a virus or viruses from human neoplasms and to find a way to prevent neoplasms in man. (The work was started in an effort to gain information on the etiology of lung cancer).

Major findings: Although work has been carried out with human tumor material, considerable effort has been expended on the SE polyoma virus. This tumor virus was recovered in tissue culture from AKR mice, and an attempt is being made to use it as a model for the recovery of a virus or viruses from human neoplasma. See Part B.

If lung cancer or any cancer is an infectious disease, there are prospects that a vaccine might be effective in preventing it. The propagation of the etiological agent is the first requisite for the preparation of such a vaccine.

VI. Project title: Pneumococci

Man years: Insignificant

Objectives: The objective of the project is to control pneumococcus typing sera. This is a minor project; only 4 lots of pneumococcus typing sera were released in 1959.

Major findings: The nomenclature of the Danish and American types were correlated in a paper jointly with Dr. F. Kauffman and Dr. E. Lung. See Part B.

The general aim of the project is to fulfill the obligation of controlling pneumoccus typing sera.

Part B included Yes ☒ No ☐ /

Part B: Honors, Awards and Publications

Publications from this project:

1. Eddy, B. E., Stewart, S. E., Stanton, M. F. and Marcotte, J. The Induction of tumors in rats by the SE polyoma virus. J. Nat. Cancer Inst. 22: 161-171 (1959).

2. Eddy, B. E., and Stewart, S. E., Physical properties and hemagglutinating cycopathogenic effects of the SE polyoma virus. Proc. of Canadian Cancer Conference 3: 307-324 (1959).

3. Eddy, B. E., Stewart, S. E., Kirchstein, R. L., and Young, R. D., Induction of subcutaneous nodules in rabbits with the SE polyoma virus. Nature, 183: 766-767 (March 14, 1959).

4. Eddy, B. E., and Stewart, S. E., Characteristics of the SE polyoma virus, a virus that inducds malignant tumors in mice, hamsters and rats and benign growths in rabbits, Am. Pub. Health Assoc. 49 (11), 1486-1493, 1959.

5. Eddy, B. E., S. E. polyoma virus, Part B. Characteristics of the virus "Advances in Virus Research." Editors Drs. Kenneth Smith and Lauffer. Academic Press, In press.

6. Eddy, B. E., Stewart, S. E. and Touchette, R. Effect of immunization of adult female hamsters on the latency of infection in offspring inoculated with the SE polyoma virus. Fed. Proc. Part 1, 18 (1): 565 (Abstract).

7. Kauffman, F., Lund, E., and Eddy, B. E., Proposal for a change in the nomenclature of Diplococcus pneumoniae and a comparison of the Danish and American type designation. Internation Bulletin of Bacteriological Nomenclature and Toxonomy. (In press).

8. Stewart, S. E., and Eddy, B. E., Properties of a tumor inducing virus recovered from mouse neoplasma. In "Perspectives in Virology." Ed. Morris Pollard. John Wiley and Sons 1959.

9. Dr. Mayorca, G. A., Eddy, B. E., Stewart, S. E., Hunter, W. ., Friend C., and Bendick, A. Isolation of infectious deoxyribonucleic acid from SE polyoma infected tissue cultures. Proc. Nat. Acad. Sci (In press).

10. Stewart, S. E., Eddy, B. E., Stanton, M., Inducation of neoplasms in mice and other mammels by a tumor agent carried in tissue culture. Proc. of Canadian Cancer Conference 3:287-305 (1959)

11. Stewart, S. E., Eddy, B. E., and Stanton, M. F., Neoplasms in rodents inducted by SE polyoma virus. Acte Union Internationale Contre le Cancer 15 (3-4): 842-851 (1959)

12. Stewart, S. E., Eddy, B. E., Stanton, M. F., and Lee, S. L., Tissue Culture plaques of SE polyoma virus. Am. A. soc. for Cancer Res. 50: April 10-12, 1959 (Abstract).

13. Stewart, S. E., Eddy, B. E., and Irwin, M. L. Tumor induction by the SE polyoma virus and inhibition of tumors by specific neutralizing antibody. Am. Pub. Health Assoc. 49 (11): 1493-1497, 1959.

14. Stanton, M. F., Stewart, S. E., Eddy, B. E. and Blackwell, R. H. The omcogenic effect of tissue culture preparations of polyoma virus on fetal mice. J. Nat. C. I. In press.

15. Stewart, S. E., and Eddy, B. E., A review of the biological properties of the SE polyoma virus. Leukemia Symposium of Hematology (Rome) (In press).

Papers of which I was the senior author and one paper which I was the second author were chosen for inclusion in the 1958, 1959 Yearbook of Cancer.

Invitation to speak at the Gordon Conferences in New London, New Hampshire. Presented a paper "Studies on the latency of SE polyoma virus infections" September 2, 1959.

Vice president and a Board of Managers of Washington Academy of Science.

Member of Executive Committee of Washington Branch of the Society of American Bacteriologists.

Serial No. DBS - 37
1. Laboratory of Viral Products
2. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:
1. Studies on variation in virulence of poliovirus IV. The effect of 5-bromouracil and stable cell lines
2. Experimental variation in mouse virulence of Echo 9 virus.
3. Studies on attenuated polio strains.
4. Intracerebral passage of sarcoma 180 in mice and hamsters.
5. Hydrocephalus in suckling mice inoculated with polyoma virus.
6. Masking and unmasking of polyoma virus: transmission of the virus from mother to offspring *in utero*.
7. Antimicrobial effect of abalone juice.

Principal Investigator: Dr. C. P. Li

Other Investigator: None

Cooperating Units: Dr. Benjamin Prescott, Bacterial Chemistry, NIAID
Drs. R. L. Kirschstein and Albert Holtz

Man Years
Total: 6
Professional: 1
Other: 5

Project Description: Studies on variation in virulence of poliovirus IV. The effect of 5-bromouracil and stable cell lines

Objective and Methods: These have been described in previous annual reports.

Work Accomplished: The 4th paper of this series of studies was published in J. Immunol. (see Part B). The 6hief finding is that when stable monkey kidney cells maintained in lactalbumin hydrolysate medium was used for culture of mouse-virulent Type III poliovirus, its mouse virulence was greatly reduced or lost after 2 serial passages. This confirmed our previous finding that the mouse-virulence of this virus depended on the type of host cell in which the virus was grown.

2. Experimental variation in mouse virulence of Echo 9 virus

The objective of this study is to see whether mouse virulence of Echo 9 virus would be influenced by the host cell in a similar way as Type III poliovirus was. Serial passages of Echo 9 virus was carried out in 2 sets of monolayer tissue culture in Eagle's medium: a) in primary monkey kidney (MK) cells b) in monkey stable kidney (MS) cells. After several passages, the virus lost most of its virulence for mice in MS cells but not MK cells, indicating that mouse virulence of Echo 9 virus could also be influenced by host cell. (See Part B).

3. Studies on attenuated polio strains

A. The MS marker of attenuated polio strains.

The formation of plaques on monkey stable kidney (MS) cells by attenuated polio strains (Type I, II, III) from 3 different sources were studied in comparison with the corresponding virulent strains. It was found that all attenuated strains formed no plaques or small plaques 1 - 2 mm in diameter after 6 days of incubation following the technique of Kanda and Melnick (J. Exp. Med. 1959 109, 9), while the corresponding virulent strains formed both large and small plaques (2-8 mm). The different attenuated strains showed difference in monkey virulence but that did not reflect on MS marker. This project is still under study.

B. The effect of photodynamic action on B virus present in live polio vaccine.

This is a joint work with Dr. C. W. Hiatt and details are described in his report.

4. Intracerebral passage of sarcoma 180 in mice and hamsters.

This is a joint work with Dr. R. L. Kirschstein continued from last year, 1958. The finding is as follows: Mouse sarcoma 180 has been through a total of more than 30 IC passages in suckling and adult mice. The IC route in suckling mice was about 1000 times as effective as the subcutaneous route in adult mice. After serial IC passages in suckling mice the sarcoma was adapted to suckling hamsters by IC injection and more than 12 serial passages have been carried out in this animal (See Part B). A strain of sarcoma 180 cells which has undergone more than 25 TC passages was also studied. It was found that the mouse sarcoma 180 harbored the polyoma virus while the TC sarcoma 180 did not.

5. Hydrocephalus in suckling mice inoculated with polyoma virus.

During the course of a study of IC passage of sarcoma 180 in mice and hamsters, suspensions of brain tissues from these animals

at various passage levels were inoculated into mouse embryo tissue cultures (METC). From these cultures a virus was isolated which was identified as polyoma virus. When the TC fluid containing this virus was inoculated IC into one-day-old, general purpose, Swiss mice from the animal production colony of the National Institutes of Health, about 30% of them developed hydrocephalus in 3 to 6 weeks. A preliminary report was published in Virology (See Part B). The question which was naturally raised is that whether some of the idippathic cases of human hydrocephalus could be due to viral infection in utero. A joint project with Dr. E. B. Spitz of Children's Hospital of Philadelphia and Drs. Albert Holtz and Kirschstein of our laboratory has been arranged. Dr. Spitz has seen more than 2000 cases of human hydrocephalus and the clinical course of his patients are more or less like that of our hydrocephalic mice. Attempt is being made to culture a virus from human hydrocephalic fluid.

6. Masking and unmakking of polyoma virus: transmission of the virus from mother to offspring in utero.

Polyoma virus was isolated several times from normal uninoculated mouse embryo tissue culture prepared from NIH general purpose Swiss mice. These mice were found by Dr. W. P. Rowe to be free of antibody against polyoma virus. The blood of a limited number of mice including pregnant ones were also tested in this laboratory and no significant HI titer was found. However, it seemed to us that the absence of antibody in mice in a given colony does not necessarily mean absence of latent infection of a tumor-inducing virus. The virus may pass from parents to offspring in a very small quantity or in a masked form, stimulating little or no antibody production, but in trypsinized TC of the embryo cells the virus could be unmakked and/or multiply.

Experiments were conducted to see whether mice can be infected with polyoma virus in utero. Adult female mice were infected before or after mating or during early pregnancy with approximately $10^{5.0}$ $TCID_{50}$ of the polyoma virus in tissue culture (TC) fluid by intracerebral, intramuscular or intraperitoneal injection. From 5 to 29 days after inoculation some of the pregnant mice were sacrificed and their embryos trypsinized for preparation of monolayer TC in roller tubes. The cultures were observed for about 2 months and the TC fluid was harvested from time to time for testing polyoma virus by the hemagglutination reaction. Out of 8 such experiments, the polyoma virus was isolated 5 times. These experiments suggested that the test mice were infected in utero. Further work is in progress.

7. Antimicrobial effect of abalone juice.

The first part of this project is completed and a manuscript has been prepared and approved for publication by DBS Editorial Committee. Further work on isolation, purification and concentration of the active factor has been done jointly with Dr. E. Prescott, of Bacterial Biochemistry, NIAID. The active factor (or factors) showed a strong antibiotic action against Staphylococcus aureus and B. subtilis, was water soluble but not dialyzable and was associated with protein or was protein in nature itself. It is therefore not an ordinary antibiotic. In reviewing the literature for antibiotic and antiviral agents, it was noticed that efforts have been largely concentrated in the field of microbial products. It is now felt necessary to shift toward some other material such as sea food. Because of the abundance and variety of organic materials in sea watter, and the known ability of gastropods to filter and concentrate the suspended solids of their aquatic milieu, certain sea animals such as abalone, may be regarded as a potential source to search for antibiotics and antiviral agents. The present experiments demonstrated beyond any doubt the extraordinary antibiotic action of the abalone juice. One of the chief advantages of its use for therapeutic purpose is that it is edible as a food, and therefore non-toxic. It is believed that this discovery will open a new chapter in medicine.

Part B included Yes /X/ No /__/

Serial No. DBS -37

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications

Li, C. P., Studies on Variation in Virulence of Poliomyelitis Virus, IV. The Effect of 5-bromouracil and Stable Cell Lines. J. Immunol. 1959, 83, 511

Li, C. P., Experimental Variation in Mouse Virulence of Echo 9 Virus, Proc. Soc. Exp. Biol. and Med, 1959, 102, 223.

Li, C. P., Kirschstein, R. L., and Jahnes, W. G., Intracerebral Passage of Sarcoma 180 in Mice and Hamsters, Proc. Soc. Exp. Biol. and Med., 1959, 101, 252.

Li, C. P., Jahnes, W. G, Hydrocephalus in Suckling Mice Inoculated with Polyoma Virus, Virology, 1959, 9, 489.

Serial No. DBS - 58
1. Laboratory of Viral Products
2. Laboratory Unit No.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Possibility of Laboratory Cultivation of Human Hepatitis Virus

Principal Investigator: Dr. Joseph P. O'Malley

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.34
Professional: 0.67
Other: 0.67

Project Description:

The phase of this project dealing with the attempts to infect fragments of human liver grafted onto the chorioallantoic membrane of the developing chick embryo with plasma known to produce hepatitis in human volunteers has been abandoned due to repeated contamination of the grafts with antibiotic resistant staphylococci.

Effort is being concentrated along the lines of screening serum specimens from humans in various stages of clinical viral hepatitis in a wide variety of mammalian and avian tissue cultures. These tissue cultures are closely examined for any cytopathogenic effects that may be elicited. These effects, when they occur, are followed up until either lack of transmissibility or identification of the agent is demonstrated.

The laboratory cultivation of human hepatitis virus remains one of the major unsolved problems of basic and medical virology. Its solution would permit the establishment of an effective method for plasma and blood sterilization and, thereby, afford greater safety from infection for anyone receiving blood or blood products.

Part B included: Yes/__/ No /X/

Serial No. DBS-39
1. Laboratory of Viral Products
2. Laboratory Unit No.
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Standardization of Measles and Mumps Neutralizing Antibody in Human Gamma Globulin

Principal Investigator: Dr. Joseph P. O'Malley

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.66
Professional: 0.33
Other: 0.33

Project Description:

Gamma globulin samples representing fractions from plasmas pooled in different years from various parts of the United States are being screened to determine their measles and mumps neutralizing antibody levels. Neutralization titers of these specimens cannot be reproduced consistently when tissue cultures with liquid supernates are employed.

Hence, this problem is now being approached from the aspect of developing plaque forming techniques for both measles and mumps viruses. These methods, if successful, will afford precise measurement of measles and mumps neutralizing antibody in the human gamma globulin specimens.

The groundwork will then have been layed for the establishment of a national standard antibody pool for measles and mumps viruses.

Part B included: Yes /__/ No /X/

Serial No. [illegible]
1. Laboratory of Viral Products
2. Research Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Measles Virus and the Serologic Response of Experimental Animals to the Virus Grown in Tissue Culture

Principal Investigator: Dr. Miklos N. Dreguss

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 2
Professional: 1
Other: 1

Patient Days: None

Project Description

Objective: The objective of this research project is to study measles virus in tissue culture and to develop serologic tests for determination of the antigenicity of the virus and for measuring the measles antibody in immune sera.

Course of Investigation: First, measles virus strains were studied by propagating them in tissue culture using various cell types. Then, the immunologic response to such virus has been investigated by inoculating experimental animals, and the resulting antibody was determined by serologic tests *in vitro*.

Methods Employed: The tissue culture method of Enders and Peebles has been adopted, with some modifications, to produce virus and prepare antigens for the serologic tests. Artificial immune sera were produced by applying live virus at various routes for immunization of animals. Among serologic tests, complement-fixation (CF) was used extensively.

Major Findings: In the earlier phase of this project a detailed study was conducted to grow measles virus in eight different kinds of mammalian cells (primary and continuous cell lines), and in primary chick embryo tissue culture cells, after adapting the virus to grow in the particular cell types. Immune serum was produced in several animal species and a basic CF-method was worked out for measuring the antibody titer of immune sera and testing antigenicity of the virus.

This year the following main objectives were selected for study: (1) Clarifying the role of part-antigen, presumably of tissue origin, present in tissue culture fluids in which the measles virus was propagated; (2) Comparative titrations of antibody obtained by the use of antigens in various tissue culture cells, grown with varied culture methods and media; (3) Persistence of antibody levels in the immunized animals; (4) Intrauterine transmission of measles antibody to the offspring of immunized guinea-pigs; (5) Persistence of the passively acquired antibody in the offspring, and in animals immunized with homologous and heterologous serum.

(1) It was demonstrated that the measles antigen behaves differently during the early and later phase of cultivation in tissue culture; in the fluid phase progressively different antigens were found. This explains some of the early controversial findings both by others and by ourselves. By multiple inoculations, the antigen of presumably tissue origin assumes an accentuated role in the resulting antibody.

(2) Comparative titrations have shown that there is good cross reaction between measles antibody on one hand, and the various measles virus antigens on the other hand, however, different types of tissue culture cells and maintenance media were used when the virus was grown. The former do not influence significantly the measles virus antibody titer. By using proper arrangements, the serologic reaction to the tissue culture components remains at low level, measured by CF-test, as compared to the serologic response directed to the virus.

(3) The antibody level was followed in a number of immunized animals for more than a year. The level of circulating CF-antibody decreases with passing of time, still it is demonstrable after one year following a single injection of live virus. The antibody level is maintained much better after two or more inoculations, but a gradual decrease is noticeable here too.

(4) Measles antibody of immunized guinea-pigs is readily transmitted diaplacentarly to the offspring and is clearly demonstrable in the serum of the latter for at least three month after birth

(5) If measles antibody is administered passively to normal guinea-pigs, its presence in the serum of the recipients may be s by complement-fixation, the titer eliciting a gradual decrease in th A study is in progress for application of this principle, in a simil fashion, to concentrated human gamma-globulin preparations which ca not be tested in native form by CF, being always anticomplementary.

Publications are in preparation to report the findings in some detail soon. The experimental results of this project may be utilized in control methods either for measuring antibody in commercial gamma-globulin preparations, or in the process of testing measl vaccines.

Part B included: Yes ☐ No ☒

Serial No. DBS-41
1. Laboratory of Viral Products
2. Rickettsial Diseases
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Immunogenesis and Pathogenesis in Animals and in Man and on Virulence Control in Animals of the E Strain of *Rickettsia prowazeki*

Principal Investigator: Dr. J. A. Morris

Other Investigators: Mr. C. G. Aulisio

Cooperating Units: Commission of Rickettsial Diseases, Armed Forces Epidemiological Board
University of Maryland School of Medicine
Walter Reed Army Institute of Research

Man Years:
Total: 1 1/2
Professional: 1
Other: 1/2

Objective: The objective is accumulation of information which may lead to standardization of procedures for preparation and assay of an attenuated living vaccine for use in prevention of epidemic typhus fever in man.

Methods Employed: Ordinary procedures used in a research laboratory engaged in studies on rickettsiae and rickettsial vaccines.

Major Findings: Members of cooperating government and university groups have met for discussions which resulted in agreement on procedures for (1) selection and preparation of a standard primary seed pool of the E strain for use in vaccine production, (2) production of vaccine in suitable lots for use in field studies in Yugoslavia in summer 1960, (3) standardization of vaccine potency and safety and (4) establishment of basic protocols of studies to determine a) occurrence of rickettsemia in animals and in man following vaccination, b) persistence of rickettsiae in tissues of vaccinated subjects and c) stability of the avirulent character of the E strain during louse transfer.

Significance of Project to DBS Program: Results of the current study may serve as basis for development of the E strain to the point of general acceptance as a biologic useful in control of epidemic typhus in man.

Part B included: Yes /__/ No /X/

Serial No. DBS-42
1. Laboratory of Viral Products
2. Rickettsial Diseases
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Procedures on preparation of a stable concentrated epidemic typhus vaccine of the Cox-type that can be stockpiled for use in national emergencies and reassessment of old and development of new assay procedures for this vaccine.

Principal Investigator: Dr. J. A. Morris

Other Investigators: Mr. C. G. Aulisio

Cooperating Units:
Commission on Rickettsial Diseases, AFEB
University of Maryland School of Medicine
Walter Reed Army Institute of Research

Man Years:
Total: 1
Professional: 1/2
Other: 1/2

Objectives: To determine a method of preparation of a stable, concentrated, epidemic typhus vaccine of the Cox-type that can be stockpiled and to devise methods to measure accurately potency of such inactivated epidemic typhus vaccines.

Methods employed: Modifications of ordinary procedures used in a research laboratory engaged in studies on rickettsial vaccines and application of a recently developed technique, i.e. fluorescent antigen-antibody systems to rickettsial vaccine assay procedures.

Major Findings: A program has been instituted to measure the serological response in human beings to concentrated, partially purified typhus vaccine prepared on a laboratory scale by Jackson and her co-workers and to assay the potency of this material by standard methods, by Jackson's modification of Kitaoka's technique and by Coon's method of conjugating antibody to fluorescent dyes

Significance to the Division: Rickettsial diseases [illegible] become of importance throughout the world following [illegible] such as might be brought about by nuclear attack. It is [illegible] of importance that steps be taken to produce and to [illegible] and potent rickettsial vaccines.

Part B included: Yes ☐ No ☒

Serial No. DBS- 43
1. Laboratory of Viral Products
2. Rickettsial Diseases
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Study of Evaluation of Factors in Animals and in Man Related to Spontaneous Activation of Latent Rickettsial Agents

Principal Investigator: Dr. J. A. Morris

Other Investigators: Mr. C. G. Aulisio

Cooperating Units: Commission on Rickettsial Diseases, AFEB

Man Years:
Total: 1
Professional: 1/2
Other: 1/2

Objective: To obtain information on basic mechanism of maintenance of causal agents of Q-fever and murine typhus in animals and in man in absence of overt disease with the idea of recovering from such hosts attenuated strains of R. burneti and R. mooseri with virulence characteristics similar to the attenuated E strain of R. prowazeki.

Methods Employed: To determine the behavior on passage to non-immune animal hosts of the recovered latent rickettsiae.

Major Findings: This is a projected study.

Significance to the Program of DBS: Selection of strains of rickettsiae useful in development of attenuated vaccines for control of rickettsioses in man.

Part B included: Yes /__/ No /X/

Serial No. DBS-44
1. Laboratory of Viral Products
2. Rickettsial Diseases
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development of Procedures to Measure Immunizing Capacity in Man and in Animals of Q-fever vaccine.

Principal Investigator: Dr. J. A. Morris

Other Investigators: Mr. C. G. Aulisio

Cooperating Units: Commission on Rickettsial Diseases, AFEB
Walter Reed Army Institute of Research

Man Years:
Total: 1/2
Professional: 1/2
Other: 0

Objectives: Development of procedures to determine minimum requirements of Q-fever vaccine and determination of stability of "Q-fever vaccine when prepared and stored under different conditions.

Methods Employed: Conventional procedures of precaution employed in a research laboratory engaged in studies on highly infectious agents of disease. Guinea-pig vaccination-challenge tests, complement-fixing antigen measurements and fluorescent antibody determinations will be used in the assay of pilot plant lots of Q-fever vaccine.

Major Findings: Discussions were held with cooperating units which resulted in agreements that pilot plant lots of Q-fever vaccine will be produced by Walter Reed Army Institute of Research. These lots will be assayed in potency by the Rickettsial Unit of DBS and will be used in experimental animal vaccination work by DBS and in human vaccination studies by cooperating Army units.

Significance to the Program of DBS: Experimental findings may serve as basis for establishing minimum requirements for Q-fever vaccine.

Part B not included: Yes /__/ No /X/

Serial No. DBS-45
1. Laboratory of Viral [illegible]
2. Testing Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Studies of the neurovirulence of Yellow-Fever (17-D) Vaccine in Monkeys.

Principal Investigator: Dr. Gerald L. Van Hoosier

Other Investigators: Dr. R. L. Kirschstein
Dr. George Hottle
Mr. B. Burks
Mr. G. Rusten

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Patient Days: None

Project Description:

Objectives: To determine whether the seed lots of Yellow Fever (17-D) vaccine as produced in the U. S. would meet proposed new WHO regulations.

Methods Employed: A total of 30 Rhesus monkeys were inoculated intracerebrally with Yellow Fever virus (strain 17-D, Lot AB1017). Twenty monkeys received 0.25 ml. of undiluted virus material and ten received 0.25 ml. of material diluted 1:25. Examination of pre- and post-inoculation sera for antibodies was carried out. Sera taken during the test period was studied for the presence of virus by inoculation of mice intracerebrally. Histopathological examination of the central nervous system, heart, liver, spleen, adrenal and kidneys was carried out.

Major Findings: Twenty-nine of 30 monkeys had no paralysis but all animals had lesions in the brains and spinal cords. None had lesions in the other viscera.

Significance to the Program of DBS: The seed lots of Yellow-Fever vaccine (17-D) as produced in the U. S. will meet the proposed new WHO regulations.

Proposed Course of Project: The project has been completed and a detailed report has been submitted.

Part B included Yes ☐ No ☒

Serial No. [illegible]
1. Laboratory of Viral Products
2. General Virology Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations concerning Measles Virus and Vaccine.

Principal Investigator: Dr. Harry M. Meyer, Jr.

Other Investigators: Dr. Ernest C. Cutchins
Dr. Juan C. Rivadeneira
Miss Nancy G. Rogers

Cooperating Units: None

Man Years:
Total: 3.0
Professional: 1 3/4
Other: 1 1/4

Patient Days: None

Project Description:

A. Experimental vaccine production and stability studies.

Several passage levels of the Edmonston strain of measles virus have been propagated in a number of host systems including embryonated eggs, chick embryo, dog kidney and other primary as well as stable line tissue cultures. Virus yields have been relatively low in all systems studied but especially so in embryonated eggs and chick embryo tissue cultures. The Edmonston strain multiplies in dog kidney tissue culture but produces little or no cytopathogenic effect (CPE). CPE in chick embryo cultures is discernable but minimal while in a number of human and simian cell cultures the CPE is prominent. The virus is markedly heat labile and has poor stability on freezing and thawing.

The goals of these studies are to achieve optimum virus production and stability on storage.

B. Studies directed toward standardization of the measles virus neutralization and complement-fixation tests.

Great variation exists from laboratory to laboratory in the performance of neutralization and complement-fixation tests in measles. Variables in the 2 tests are under study in order that

recommendations leading to standardization of methodology can be made.

Such uniformity should aid in correlation of data obtained from different laboratories.

C. Studies concerned with preparation of measles reference antiserum.

No measles reference antiserum is available at present. Various methods of antiserum production are currently being evaluated and on completion of these studies it should be possible to make specific recommendations leading to procurement of a large pool of reference immune serum. In addition to serving as the standard reference for the control of neutralization and complement-fixation tests employed in active immunization studies this preparation would serve as a guide to the potency of human gamma globulin pools for use in passive immunization against measles.

D. Evaluation of various features of attenuated and virulent measles strains in search for "markers" of attenuation.

At present the only known way to discern virulent from attenuated measles strains requires the inoculation of either susceptible humans or primates; therefore, it is obvious that in vitro methods are badly needed. Both virulent and attenuated measles strains are currently under study in search for possible "markers" of attenuation.

E. Comparison of methods available for the primary isolation of virulent measles virus and recovery of tissue culture adapted and attenuated measles strains.

Existing laboratory strains of measles virus have been obtained largely through isolation from clinical materials using primary tissue culture preparations either of human kidney, human amnion or monkey kidney. Little is known about the comparative sensitivity of these preparations or of various continuous cell lines and susceptible animals in the detection of naturally occurring measles strains.

In similar fashion no extensive attempts have been made to evaluate the various methods available for the recovery of either virulent or avirulent tissue culture adapted strains.

Studies directed toward resolving these problems are in progress.

Part B included Yes ☐ No ☒

Serial No. DBS-47
1. Laboratory of Viral Products
2. General Virology Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations Concerning Lymphocytic Choriomeningitis (LCM) Virus

Principal Investigator: Dr. Harry M. Meyer, Jr.

Other Investigators: Miss Nancy G. Rogers

Cooperating Units: None

Man Years:
Total: 1/6
Professional: 1/6
Other: 0

Patient Days: None

Project Description:

Evaluation of methods available for the detection of LCM virus.

The accepted method for detection (Safety testing) of LCM virus in vaccines prepared from monkey kidney tissue cultures involves intracerebral inoculation of ten mice with the inactivated vaccine under test. This procedure has been of a far from critical nature thus far since the formaldehyde treatment employed to inactivate poliomyelitis and adenoviruses in vaccines would in all likelihood inactivate any LCM virus present. With the advent of "live" virus vaccines prepared from animal tissues this inherent safety factor will no longer be present.

An evaluation of methods available for the detection of small amounts of LCM virus is being made. Successful conclusion of this line of investigation could lead to modification of the currently accepted safety test.

Part B included Yes ☐ No ☒

Serial No. DBS-[illegible]
1. Laboratory of Viral Immuno
2. Research Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations Concerning Vaccinia Virus and Vacc

Principal Investigator: Dr. Ernest C. Cutchins

Other Investigators: None

Cooperating Units: Laboratory of Control Activities (A 1 and 3)
Employees Health Service (B)
Division of Immunology, Walter Reed Army Institute of Research (A 2)

Man Years:
Total: 1 5/6
Professional: 1/2
Other: 1 1/3

Project Description:

A. Assay of smallpox vaccine in cell cultures.

1. Assay of commercial smallpox vaccine in cultures of monkey and rabbit kidney cells.

Objectives: Titration in cell culture as a procedure for the potency assay of smallpox vaccines has been suggested previously. The feasibility of this suggestion required the titration of a number of vaccines from different sources in selected cell cultures to establish what levels of infectivity, by this method, should be expected of commercial preparations.

Methods: Vaccines were titrated in monkey kidney cells by the plaque technique and in tubes of rabbit kidney cells for tissue culture $dose_{50}$ determinations (TCD_{50})

Major Findings: Thirty-six commercial smallpox vaccines have been assayed for infectivity in these 2 cell culture systems. Of these, only 6 had mean plaque forming unit (PFU) titers of less than $10^{7.5}$ TCD_{50} per ml in monkey kidney cells and nine had titers of less than $10^{7.5}$ TCD50 per ml. in rabbit kidney cells. When assayed by the traditional rabbit scarification method (Dr. Kolb, Laboratory of Control Activities) 5 of the 36 vaccines

found to be of inferior potency. Only 2 of these 5 were included among the 6 vaccines having titers of less than $10^{7.5}$ PFU per ml and 3 were among the 9 with titers of less than $10^{7.5}$ TCD_{50} per ml. The remainder of those vaccines of inferior potency by rabbit scarification had cell culture infectivity titers as high or higher than those found acceptable by the scarification method.

2. Stability of dried smallpox vaccine as indicated by long term assay in cell cultures.

Objectives: To study - 1. The feasibility of titration in cell cultures as a procedure for the potency assay of smallpox vaccines, and, 2. The cell culture infectivity of dried smallpox vaccine following storage at various temperatures.

Methods: Ampoules of a single lot of dried smallpox vaccine were stored at -20C, +4C, +22C, +37C, +45C and +56C. At selected intervals samples of the vaccine stored at these temperatures were titrated in monkey and rabbit kidney cells. Simultaneously the same samples were assayed by rabbit scarification (Mr. Kolb, Laboratory of Control Activities) and by inoculation on the chorioallantoic membrane, in rabbit kidney cells and in certain instances in humans (Walter Reed Army Institute of Research).

Major Findings: Ampoules of the dried vaccine stored at -20C and titrated at each of the periodic assays have served as a control for the study. The mean titer of 38 ampoules in 19 assays was $10^{8.1}$[illegible] PFU per ml. in monkey kidney cells. At the most recent assay, after one year of storage, the mean PFU per ml titer of 2 ampoules stored at the temperatures indicated were as follows: +22C, $10^{8.03}$; +37C, $10^{7.56}$; +45C, $10^{6.98}$; and +56C, $10^{5.92}$. In rabbit kidney cells considerable variation has been noted in the titer of the control from one assay to another. The average titer of 30 ampoules in 15 tests was $10^{3.05}$ TCD_{50} per ml. with a minimum of $10^{7.37}$ and a maximum of $10^{8.60}$. At the most recent assay, after 11 months of storage, the titers of each of 2 ampoules were as follows: +22C, $10^{7.33}$ and $\geqslant 10^{8.0}$; +37C; $\geqslant 10^{7.5}$ and $10^{7.40}$; +45C, $10^{6.28}$ and $10^{6.}$[illegible]; and +56C, $10^{4.74}$ and $10^{4.86}$.

B. Antibody response to smallpox vaccination as measured by a cell culture plaque reduction technique.

Continuing the study of vaccinia neutralizing antibody resulting from smallpox vaccination, approximately 50 adults with varying histories of smallpox vaccination have been bled prior to and 1 month following revaccination with a single lot of smallpox vaccine. Dermal reactions to revaccination were recorded (Dr. [illegible] Employee's Health Service). These sera are being examined currently

for vaccinia neutralising antibodies in a test in which the antibody content of a serum is indicated by its ability to reduce by 50% the number of plaque forming units of a vaccinia virus suspension following incubation of virus-serum mixtures.

Part B included Yes /__/ No /X /

Serial No. DBS-62
1. Laboratory of Viral Products
2. General Virology Unit
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Investigations Concerning Poliovirus and Vaccines

Principal Investigator: Dr. Frank T. Perkins

Other Investigators: Dr. Ernest C. Cutchins

Cooperating Units: None

Man Years:
Total: 2 1/6
Professional: 1/2
Other: 1 2/3

Patient Days: None

Project Description:

A. Further investigations on the Neutralizing Antibody Combining Test as a method to determine the antigenic content of killed poliomyelitis vaccines.

The neutralizing antibody combining test, as described by Benyesh and Melnick (Bull. Wld. Health Org. (1959) 20, 1075), has been used for determination of the potency of "killed" poliovirus vaccines. Since the project was initiated, 79 vaccines have been tested. Eleven vaccines have been tested during a storage period of 14 months and very little loss in potency has been observed. While to casual inspection there appears to be considerable agreement among results from repeated tests on any one vaccine the degree of reproducibility of the test has not been fully explored. Thus in practice, it might be difficult or impossible to distinguish between two vaccines with potencies in the same general range. Potency values obtained by this method do not correlate well with results from chick or monkey tests.

Further studies with the neutralizing antibody combining test, however, are continuing, together with the monkey and chick potency tests, to assay the antigenicity of the three components of inactivated poliomyelitis vaccine. The serological response of children to the vaccines selected for the laboratory tests will also be measured in an attempt to correlate the laboratory findings with the field results. In this study the potency of the vaccine is being measured in both the field and laboratory concurrently.

B. Study of the kinetics of neutralization of various polioviruses to attempt differentiation of attenuated from virulent strains.

Attempts are being made to find a test system in which poliovirus strains will be effectively neutralized by homologous sera and heterologous strains will be incompletely neutralized. Two approaches are being made: in the one, the rate of virus neutralization is compared for both the homologous and heterologous systems, whereas in the other, dilutions of virus and serum are made in an attempt to achieve complete neutralization in the homologous system and incomplete neutralization in the heterologous systems.

Virus pools have been harvested from both monkey kidney and HeLa cell cultures in an attempt to minimize non-specific reactions.

Antisera have been produced in monkeys from virus pools harvested from monkey kidney cells and in rabbits from the HeLa cell harvests. The greatest promise of success thus far has been obtained in tests in which neutralization is just complete in the homologous system and incomplete in the heterologous systems.

C. Collaboration in an international serological study designed to establish poliomyelitis standard reference antisera.

An international study designed to determine the potency of six test sera for each virus type in terms of monospecific standard sera has been initiated by the World Health Organization. This laboratory has participated in the study and the sera have been titrated by both the cytopathic and metabolic inhibition tests on each of four test days in which cells from different monkey kidneys were used.

Part B included: Yes /__/ No /X/

Serial No. DBS - 50
1. Laboratory of Blood & Blood Products
2. Blood Bank Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development and Application of Immunohematological Research Procedures.

Principal Investigator: Dr. Paul J. Schmidt

Other Investigators: Dr. Allan Kliman
Mrs. Eleanor G. Morrison
Mr. Webster C. Leyshon
Mr. Robert C. Scheno
Dr. Jane Shohl
Mr. Joel M. Solomon
Dr. Mitsuo Yokoyama

Cooperating Units: None

Man Years:
Total: 1.9
Professional: 1.5
Other: 0.4

Patient Days: None

Project Description:

Objectives: The objectives of the project are to search for the rare antibodies to the human blood factors which result from the antigenic stimulus of pregnancy, transfusion, and natural exposure; to develop better methods for this work and its application to clinical and laboratory medicine.

Methods Employed: (a) Blood samples from random blood donors, transfusion recipients, anthropological and disease groups, as well as patients presenting specific problems are screened for rare antigens and antibodies using tools and techniques developed in this laboratory as well as those exchanged with other workers.

(b) Intensive study is made of specific findings from the above screening procedures for comparison with known antigenic blood components with the aim of making recommendations for specific therapy in individual cases and for broad guide lines for biologics control.

(c) The response of normal individuals to natural exposure to blood group substances and the modification of this response by therapy is being established as a baseline for the evaluation of the clinical picture of antibody response.

(d) The serological characteristics of complex antibodies of the Rh system are being intensively studied by standard hemagglutination, absorption, and elution methods.

(e) The effects of ionic strength, incubation time, and composition of medium upon agglutination reactions are being investigated.

(f) The immunological and chemical properties of fractions collected after ion exchange fractionation of anti-human serum is being determined. Human globulins are injected into animals to produce anti-human sera; these sera are studied in turn by gel diffusion plates and various electrophoretic techniques.

(g) Rabbits and chickens are being used for the production of antisera to rare blood group antigens. These antisera are studied by electrophoretic methods in addition to absorptive, elution, and inhibition techniques.

Major Findings: (a) The screening techniques in use in this laboratory have resulted in the discovery of the second person known to have the rh^G factor. This blood type variant is of basic interest in the study of the Rh factor and the red cells involved are being used as a standard in further investigations. Over 1200 complete genotypes have been performed on anthropological and disease groups to establish the incidence and mode of inheritance for the long-term storage of antigen-antibody complexes of isolated cases for simultaneous comparison.

(b) Intensive study of individual cases has demonstrated the first known incident in which the rare A_x factor was involved in the clinical response to transfusion. Specific recommendations were made which are applicable to the routine preparation of blood for transfusion.

(c) Preliminary analysis of the almost completed data on possible serial variations in antibody titers resulting from natural stimulation indicates that this is not a constant finding. The effect of various modes of therapy for unrelated diseases, on the other hand, may require controlled analysis for each therapeutic agent.

(d) Data have been accumulated which indicate that red cells of phenotypes rh^G, c^v, and rh'^N closely resemble each other in their inability to be agglutinated by anti-rh_i. The differences which this antibody recognizes have been evaluated by means of dosage effect titrations.

(e) Preliminary results show a definite dependence of reactivity in agglutination reactions upon ionic strength and the chemical components of the reaction medium.

(f) Corresponding fractions from ion exchange fractionation of specific antisera of the ABC blood group system show different characteristics and activities by immunochemical analysis.

(g) The production of antisera in animals is underway, but there are no results to date.

Significance to the Program of DBS: The transfusion of blood from one person to another, although performed over 5,000,000 times per year in this country alone, is a unique situation of biological exposure to foreign antigens. Knowledge of the effects to be expected from these transfers can assist this Division in determining the safeguards to be applied through licensure. This knowledge is being gained by direct observation of donor and recipient polulations

Proposed Course of Project: (a) Screening will continue to be applied to random samples. Analysis will begin of the expected correlations to be made between blood groups and disease and blood groups and heredity. Study will begin of immunochemical methods.

(b) Specific identification of rare bloods will continue with a view to stockpiling rare red cells and antibodies for use in establishing standards.

(c) Analysis of serial isoagglutinin determinations will be made and baseline data will be established.

(d) The study of antibodies of the Rh system will be extended to include anti-hr' and anti-hr".

(e) The investigations covered in paragraphs e, f, and g, all just started, will continue.

Part B included Yes /X/ No /__/

Serial No. DBS-50

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Kevy, Sherwin V. and Morrison, Eleanor. The Stability, after Frozen Storage, of an In Vivo Antigen-Antibody Bonding on Red Blood Cells. Am. J. Clin. Path., 31:38-41, Jan. 1959

Schmidt, Paul J. and Chaplin, Hugh, Jr. A Modified Sterile Pilot Tube for Collection of the Donor's Blood for Crossmatching, A Follow-up Study on the Effects of Storage. Am. J. Clin. Path., 31:362, 367, April 1959.

Schmidt, Paul J., Nancarrow, John F., Morrison, Eleanor, and Chase, Charles. A Hemolytic Reaction Due to the Transfusion of A_x Blood. J. Lab. & Clin. Med., 54:38-41, July 1959.

Kevy, Sherwin V., Schmidt, Paul J., and Leyshon, Webster C. A Second Example of the Blood Type "rh^G." Vox Sang., 4:257-266, Sept. 1959.

Serial No. DBS-51
1. Laboratory of Blood & Blood Products
2. Blood Bank Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Stability and Potency of Formed Blood Elements.

Principal Investigator: Dr. Paul J. Schmidt.

Other Investigators: Dr. John S. Fordtran
Dr. Allan Kliman
Mrs. Eleanor G. Morrison
Dr. Jane Shohl
Dr. John T. Tripp

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.6
Other: 0

Patient Days: None

Project Description:

Objectives: To establish standards of potency for the antigenicity and viability of the formed blood elements.

Methods Employed: (a) Erythrocytes with the Rh_o variant (D^u) blood factor are being tested for antigenicity in transfusion to selected recipients.

(b) The effects of extending the storage time of heparinized whole blood (Human) for use in extra-corporeal circulation are being observed.

(c) Experimental blood products with hemostatic activity are being produced for the treatment of acute bleeding episodes.

(d) Developmental research is underway on the extraction of glycerol from erythrocytes with a view towards improving the methods in use for stockpiling rare frozen red cells for transfusion.

(e) The properties of white blood cells when transfused into selected recipients are being studied by the transfusion of blood

fractions with and without white cells. The auto-antigenicity of white cells is being studied.

(f) The relationship between methods of blood collection and preservation and some of their physical characteristics is being established.

Major Findings: (a) Over 60 transfusions of Rh_o variant (D^u) blood have been given. No antigenicity of these selected units has been demonstrated.

(b) Although it was at first thought that the blood used in extracorporeal circulation must be as fresh as possible in order to duplicate the natural circulation, no clinical ill effects have been reported from heparinized whole blood (Human) preserved for 48 hours. Since the work of other investigators has shown that platelets are not viable after 24 hours of storage, it is evident that viable platelets are not necessary in the type of extracorporeal circulation used.

(c) The project on separated hemostatic formed elements is getting underway. It has been demonstrated that repeated plasmapheresis with conventional systems is practical.

(d) An increasing stockpile of rare red cells is being built for transfusion needs. Cells for transfusion have been supplied in quantity for a patient with a blood production defect with no ill effects. Rare red cells have been supplied on demand.

(e) Identification has been made of auto-immune processes in leukopenic patients. The treatment of leuko-allergic patients with white-cell-free blood fractions is being attempted.

(f) Properties of red cells due to the use of phenothiazine as a preservative have been shown to be due to a surface effect. Demonstration has been made of the physical characteristics of preserved chelated blood.

Significance to the Program of DBS: The standard of potency of blood products which it is the duty of this Division to establish must be based on experience in handling these products before applications are considered for license. Experience gained in establishing viability requirements for such products as heparinized whole blood (Human), frozen red cells, etc., are directly applicable to the Division functions. In addition, the evaluation of the antigenicity of the Rh_o variant (D^u) and leukocytes will determine to what extent this is clinically significant and in need of control.

Proposed Course of Project: (a) Experimental Rh_o variant (D^u) transfusions will be continued until a significant number are given and a tally of data can be made.

(b) Experience with the clinical effects of preserved heparinized whole blood (Human) will be analyzed for specific recommendations.

(c) Continued use of experimental blood fractions will depend on the results of screening presently being made.

(d) The stockpile of rare red blood cells will be extended and made available as needed.

(e) The progress of the work on leukocyte antibodies is being delayed by the rarity of these cases.

(f) Preparation is being made to establish in this Section the facilities to perform the radioactivity measurements necessary to the testing of effect of preservatives on the viability of the formed elements.

Part B included Yes /X/ No /_/

Serial No. DBS-51

PHS-NIH
Individual Project Report
Calendar Year 1959

Part B: Honors, Awards, and Publications

Publications other than abstracts from this project:

Freireich, Emil J., Schmidt, Paul J., Schneiderman, Marvin A., and Frei, Emil, III. A Comparative Study of the Effect of Transfusion of Fresh and Preserved Whole Blood on Bleeding in Patients with Acute Leukemia. N. Eng. J. Med., 260:6-11, Jan. 1959

Shohl, Jane, and Schmidt, Paul J. The Relationship Between Plasma Proteins and Red Cell Sedimentation in a Promethazine System. J. Lab. & Clin. Med., 54:42-48, July 1959.

Schmidt, Paul J., Morrison Eleanor G., and Shohl, Jane. The Antigenicity of the Rh_0 Variant (D^u) in Transfusion Practice (A Preliminary Report. Proceedings of the 7th Congress of the International Society of Blood Transfusion, Rome, 1958, 569-571, (S. Karger, Basel, 1959).

Serial No. DBS-52
1. Laboratory of Blood & Blood Products
2. Blood & Blood Derivatives Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Stability and Potency of Blood Fractions.

Principal Investigators: Dr. John T. Tripp
Dr. Richard T. Suchinsky

Other Investigators: Dr. David L. Aronson
Miss Ann L. Dayton
Mr. Paul O. Fehnel
Dr. John S. Finlayson
Dr. Sandor S. Shapiro

Cooperating Units: None

Man Years:
Total: 4.9
Professional: 2.9
Other: 2.0

Patient Days: None

Project Description:

Objectives: To delineate the changes occurring in the physical and chemical properties of various human plasma proteins resulting from the physical and chemical treatment incidental to processing and to investigate the properties, the mechanisms of action, and methods of assay of blood coagulation components and related systems.

Methods Employed: (a) Parallel samples of liquid and dried normal serum albumin (Human) are stored at 32°C., 5°C., and ambient room temperature. At regular intervals the materials are tested for stability by viscosity determinations, ultracentrifugation, electrophoresis, and nephelometry.

(b) A study of the effects of physical and chemical treatment of plasma derivatives is being carried out. These products have been subjected to heating to 60°C., ultraviolet irradiation, or long-term storage.

(c) To gain further insight into the clotting mechanism, changes in the biologic activity of certain clotting factors are being investigated at oil/water interfaces.

(d) A study of the effect of bovine fibrinolysin on fibrinogen utilizes routine clotting methods. The feasibility of utilizing this reaction as an assay for fibrinolysin is being investigated.

(e) The sensitivity of dry proteins to ionizing radiation appears to be a direct function of molecular size. This suggests a possible way to study molecular weights in impure systems. These target volume methods are being utilized to study molecular weights of blood clotting substances, in particular, thrombin, prothrombin, and the components of thromboplastin.

(f) Starch gel electrophoresis is being used to study prothrombin.

Major Findings: (a) The stability measurements on albumin stored for five years show that definite changes of unknown clinical significance take place in albumin stored at temperatures over 5°C. The results to date have permitted the extension of the dating period of albumin stored at refrigerator temperatures.

(b) The use of cellulose chromatography gives well-defined, reproducible results in the analysis of protein mixtures. The method has proved capable of detecting changes in albumin not readily found by other techniques and shows promise of becoming a potent tool in the analysis and isolation of trace components in protein mixtures. This technique is now being applied in the investigation of the changes noted in plasma proteins after physical treatment.

(c) The study of clotting factors at oil/water interfaces has just been initiated; so far, the main work has been the purification of the desired clotting components.

(d) Fibrinogen decay in an all bovine system appears to occur as a direct consequence of the action of fibrinolysin; however, at the moment it is also possible that part of the apparent fibrinogen decay is due to interference by lytic products with the further action of thrombin.

(e) Utilizing the target volume method, the molecular weight of thrombin has been determined at 32,000 when dried from the frozen state and 64,000 when samples are evaporated from the liquid state. The molecular weight of prothrombin has been found to be

65,000. Preliminary determinations on antihemophilic globulin give a molecular weight of about 140,000 and for plasma thromboplastin component, a molecular weight of about 90,000.

(f) A hitherto unidentified band in normal plasma has been associated with prothrombin. This band is lacking in normal serum and apparently in plasma of patients on dicumarol therapy. It has been possible to recover prothrombin activity from this band.

Significance to the Program of DBS: All Blood and blood products controlled by this Division are required to be stable and potent. Improved methods for the evaluation and assay of these characteristics *in vitro* are being developed in order to improve our ability to determine stability and potency and to place these control functions on a foundation of knowledge gained through research.

Proposed Course of Project: The various parts of this project will be continued.

Part B included Yes ☐ No ☒

Serial No. DBS-53
1. Laboratory of Blood & Blood Products
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title: Development and Application of Control Procedures for Blood and Blood Products.

Principal Investigator: Dr. John T. Tripp

Other Investigators: Dr. David L. Aronson
Miss Marietta Carr
Miss Ann L. Dayton
Mr. Joel M. Solomon
Dr. Richard T. Suchinsky

Cooperating Units: None

Man Years:
Total: 2.7
Professional: 1.7
Other: 1.0

Patient Days: None

Project Description:

Objectives: To develop improved control procedures and standards by evaluating the present control procedures and manufacturing methods as these effect the safety, purity, and potency of biologic products derived from blood.

Methods Employed: (a) Colored photographs of blood mixed with antisera are mounted in plastic slides for interpretation by blood bank technicians. The technicians record their results in different ways, utilizing their routine forms.

(b) Bacterial contamination in blood and derivatives of blood is being studied in two ways. First, in order to determine the mode of action of bacteriological filter pads and filters, these materials are immobilized by collodion imbedding to allow thin sections to be taken for microscopic examination. Second, rates of growth of recognized contaminants of whole blood are being followed.

(c) To increase the efficiency and precision of control tests requiring serial dilutions, an improved mechanical diluter was designed and evaluated.

(d) An investigation of the efficiency and applicability of shipping containers for blood and other materials requiring storage at low temperatures is being pursued by simulated and actual shipments under a variety of temperature conditions.

(e) Rabbits are injected with whole pollen solutions in an attempt to produce improved specific antisera. This study may make it possible to investigate further the possible role of allergy in post-transfusion reactions.

(f) To investigate and compare the equilibria of hemagglutination reactions of the ABO blood group system, percentages of cells left unagglutinated by mono- and multispecific antisera after various periods of time are determined hemocytometrically.

(g) In order to define the needs for new standards for anti-human sera, the reliability of unselected anti-human sera in detecting sensitization of red blood cells to isoantibodies is being studied. Routine methods employed in blood banking are used with adequate controls.

(h) Changes occurring during storage of plastic blood containers and accessory equipment are studied using _in vivo_ and _in vitro_ methods to detect these changes. At this point, infrared spectrometry has been the only method utilized.

Major Findings: (a) The study of blood banking errors has not been tabulated as yet. So far, about 100 blood bank technicians have made approximately 40,000 readings.

(b) There have been no major findings to date in the filter investigation. The study of contamination in whole blood was completed and the results are now being assembled for publication.

(c) As a result of further improvements in the instrument, a working proportional diluter has been perfected and is in use.

(d) An efficient and economical shipping container developed as a result of this study is now in routine use for the shipment of heat labile materials. Some progress has been made in the study of containers for the shipment of frozen samples and work has just started on portable refrigeration by a light-weight mechanical device.

(e) Results in the study of allergenics so far have been negative. Utilizing precipitin ring tests, agar diffusion plates, and capillary tube tests, no antibody has been detected as yet.

(f) In the study of the quantitation of antibodies, it has been found that although monospecific anti-A and anti-B will reach a true equilibrium with A_1 and B cells, the multispecific antibody of Group O sera will not. This mixed agglutination shows that absorption is not entirely effective in removing cross-reactive antibody which may conceivably play a role in blocking the equilibrium.

(g) Anti-human sera are currently produced that will detect sensitization of newborn erythrocytes to antibodies of the ABO system. Additional control criteria appear to be not only desirable but essential for the widest application of these reagents.

(h) The study of changes in plastic blood containers has barely passed the planning stage. There have been no significant findings to date.

Significance to the Program of DBS: Control procedures of greater significance and specificity can result from investigations such as those carried out as part of this project.

Proposed Course of Project: All parts of this project will continue along the same lines.

Part B included Yes / / No /X/

Calendar Year 1959

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

OFFICE OF THE DIRECTOR

CC-01
Serial No.

The administration of the Clinical Center is responsible for furnishing all services, except physician care, required for the admission, diagnosis and treatment of patients accepted to participate in the clinical investigation programs of the seven Institutes. In fulfillment of the primary mission of conducting integrated laboratory-clinical research programs, patients are provided with care of the highest quality. A major objective is to maintain an environment in which every patient receives individual personal attention, with genuine warmth and earnest interest in the patient's welfare on the part of all members of the staff. This responsibility involves continuous evaluation of activities in all hospital departments supplying the multiple services needed directly and indirectly in the care of research patients.

The ever-present scarcity of nurses has affected the Clinical Center as well as all other hospitals. As part of a series of surveys on the utilization of resources, we have embarked on a group of studies aimed at extending the time of graduate nurses available for professional duties through the possible use of (1) non-professional administrative assistants on the nursing units and (2) electronic recording and monitoring devices in the intensive care of post-surgical patients.

The past year has seen the completion of planning and the beginning of construction of the New Surgical Wing, which includes many innovations in the design of surgical facilities for neurosurgery and cardiac surgery research.

The availability of "normal control" patients has been of major importance in the conduct of certain investigations in physiology, pharmacology, psychology, and other special areas. The sources of recruitment have been augmented and volunteers are now being obtained from a well-known college, in addition to religious organizations. The participation of these normal, healthy patients is scrutinized meticulously in a review of every project by the (1) Clinical Research Committee, (2) Medical Board, (3) Office of the Director of the Clinical Center, and (4) Office of the Director, National Institutes of Health.

Admission and Occupancy Data

The calendar year 1959 was the second year during which full utilization has been achieved of the 516 bed complement of the Clinical Center. There were 3,452 admissions during the year which was an increase of 152 patients over the previous year. The average daily census through the year was 384 patients, an average occupancy rate of 74%.

Since the inauguration of the Clinical Center in 1953, over 15,000 patients have been admitted to the Clinical Center. This increase has been gradual and has shown a twenty-fold increase per year over the 176 patients admitted during the first year of occupancy. Each of these patients was referred by his own physician or medical agency and each was accepted because his diagnosis and current status suited the precise requirements of specific studies being conducted at the Clinical Center at the time of his admission.

For each patient admitted nine referrals were formally reviewed and rejected as not being suitable for one of our study projects.

The referral of patients by practicing physicians remained at a satisfactory level. The dissemination of information concerning the changes in programs of clinical investigations was carried on through publications of supplements to the booklet "Current Clinical Studies and Patient Referral Procedures" of the National Institutes of Health, in medical journals, professional "newspapers" and booklets, such as the Journal of the American Medical Association, Medical News, Scope, Resident Physician, Medical Economics, etc. Publication of the proceedings of staff conferences in the Annals of Internal Medicine has also proved helpful. Meetings with medical groups, such as the Committee on Federal Medical Services of the American Medical Association, have resulted in better understanding of the role of the Clinical Center in the care of certain types of patients.

During the past year the objective of maintaining widespread sources of referral of patients was continued. Approximately half of our patients came from areas outside of Maryland, D. C., and Virginia.

Summary:	1958	1959
Total number of admissions	3,300	3,452
Average daily patient census	384	384
Percentage of bed occupancy	74	74
Average hospital days per patient	44	41
Visits to Admissions & Followup Department	24,541	25,070

Advanced Training of Physicians

In pursuit of the major mission of research, the Clinical Center also conducts formal advanced training programs for young physicians in certain medical specialties. The Clinical Associate and Residency training programs serve the dual purpose of maintaining high quality of care for patients and augmenting the supply of physicians trained in clinical research methodology. In 1959 there were several hundred applicants from the leading hospitals in this country for these house staff positions. Approved Residency training programs are conducted in Clinical Pathology, Anatomical Pathology, Diagnostic Radiology, Internal Medicine, Neurology, Psychiatry, Ophthalmology, and Neurological Surgery. It has been the policy of several Medical Specialty Boards to accept all or part of the time served at the Clinical Center by junior members of the staff as training credit for specialty certification. This recognition of the quality of experience in our clinical programs is a significant factor in the recruitment and retention of staff.

Medical Board

The Medical Board of the National Institutes of Health functions as does the Senior Medical Committee of a university hospital. The Medical Board establishes standards and recommends policies in the care of patients participating in the research activities of the Clinical Center. The Board is composed of representatives from each of the seven Institutes as well as from the Clinical Center. The Board has several functioning committees: (1) a Clinical Research Committee which must pass on all projects involving "normal control" volunteers; (2) a Medical Record Committee which continually surveys and evaluates medical records kept on patients in the Clinical Center; (3) a Steering Committee which considers special problems pertaining to patient care; (4) a Credentials Committee which approves the qualifications of consulting physicians; (5) a Tissue Committee which studies the correlation of the pathologists' reports of tissues removed from surgery patients with the clinical diagnosis; (6) an Education Committee which advises on matters relating to postgraduate medical education conducted at the Clinical Center; (7) a Normal Controls Committee which provides advice to the Medical Board on the recruitment, utilization, and other areas of interest involving "normal control" patients.

The semi-monthly meetings of the Medical Board dealt with a large variety of subjects of significant importance in the improvement of care of our patients, maintenance of esprit d'corps of professional staff, and continuance of good rapport with referring physicians.

Information Office

The Information Office of the Clinical Center is responsible for all public information releases concerning the medical care and clinical research activities of the Clinical Center. An important component of the information services has been the assistance rendered to science writers, reporters, magazine editors, and radio and television authors in the preparation of material for the general public. Illustrative of articles appearing in the lay press are:

"Unsung Youths Who Test Untried Drugs" by Michael O'Neill--N. Y. Sunday News, June 7, 1959. A comprehensive, illustrated article on the contribution of the Clinical Center's normal volunteer program to the progress of medical research.

"God's Quiet Heroes" by Theodore Irwin--Coronet Magazine, January 1959. Article on the Church of the Brethren Volunteer Service with major emphasis on the volunteers who have participated in medical research at the Clinical Center.

"Are You Building Up a Sleep Debt?" by Theodore Irwin--Reader's Digest, January 1959. A popular article concerning the effects of sleep deprivation with reference to clinical research conducted at NIH.

"Volunteers for Service" by Harry Edward Neal--Sunday Digest, September 20, 1959. A short story about the normal volunteer program at NIH, with specific reference to the participation of two young people from the Mennonite and Brethren churches.

Another major responsibility is the editorial function relating to the publication of certain contributions to the professional literature. Examples during the past year are:

1. Manual for Metabolic Balance Studies.

   A technical handbook for the guidance of nursing staffs of other research institutions.

2. Combined Clinical Staff Conferences of the Clinical Center for the Annals of Internal Medicine:

   "Waldenstrom's Macroglobulinemia" - Vol. 50, No. 4:1010, April 1959

   "Chronic Aphthous Stomatitis, Herpes Labialis and Related Conditions" - Vol. 50, No. 6:1475, June 1959

   "Idiopathic Hypoalbuminemias" - Vol. 51, No. 3:553, September 1959

   "Sarcoidosis" - Vol. 51, No. 6:1356, December 1959

   "Amyloidosis" is scheduled for the March 1960 issue.

3. Quarterly Review of Pediatrics:

   'Research in Diseases of Childhood at the National Institutes of Health" - Vol. 14, No. 4:235, October-December 1959

4. Building Medical Research Manpower, National Institutes of Health. A new 16-page printed publication, issued February 1959.

5. Nursing at the Clinical Center, PHS Publication No. 468, revised and expanded in November 1959.

6. A Conservative Approach to Clinical Endodontic Treatment, a 32-page manual, issued in November 1959.

Scroll and Anthem:

The motif in the design of the Clinical Center is the pool at the front entrance, symbolic of the Biblical healing Pool of Bethesda. An illuminated scroll, incorporating verses 2, 3 and 4 from the Fifth Chapter of the Gospel according to St. John has been placed as a permanent display in the lobby of the Clinical Center as a reminder of our mission and obligations. In addition, a hymn incorporating these verses has been written for N.I.H. It was presented for the first time at the Christmas program for patients and staff.

Visitor Program

A steady stream of architects, clinicians, engineers, hospital administrators, health officials, nurses, nutritionists, laboratory scientists, social scientists, science teachers, and university officials comes to the Clinical Center from various parts of the U.S., Europe, Asia, Africa, Canada and Latin America. We have had an increasing number of visitors from Russia. Two of the visitors from Moscow included Dr. Alexandra M. Markov, Professor and Member of the Collegium, and Dr. Valerie N. Butrov, Counsellor of the Ministry of Foreign Affairs, who represented Mr. Nikita S. Khrushchev, Chairman of the Council of Ministers, U.S.S.R., who had originally planned to visit the Clinical Center but was obliged to cancel at the last moment.

The N.I.H. serves as a center for meetings of national and international interest. Examples during 1959 are:

Current Research in Endocrinology, sponsored by the American College of Physicians.

Special meeting for MEDICO (Medical International Cooperation).

25th Anniversary Meeting of the Society for Research in Child Development.

Micro-Circulatory Conference.

Second Pan-American Congress on Rheumatic Diseases.

Conference on Bone Marrow Transplants.

Symposium on Preventive Medicine and Health Mobilization, sponsored by the Committee on Medical Education for National Defense.

Cancer Information Conference for High School Science Teachers.

Council on Medical Television.

Conclusion:

The Clinical Center has completed its sixth year of operation as a unique organization for the advancement of laboratory research, clinical investigation, and the care of the sick. There have been many problems in the development of a highly complex institution involving a great variety of professional, technical, and administrative disciplines which are being met with earnest effort and sincere cooperation. But the concept of the "fecundity of aggregation" in bringing together creative intellects in many fields of health sciences is beginning to bring results of significant value in the conquest of disease.

Attachment A

Serial No. CC-01

CLINICAL RESEARCH COMMITTEE

Research Projects Approved, January 1, 1959 - December 31, 1959

*Title	Investigators	Institute
*Use of Normal Volunteers in the Study of Effects of Intravenous Infusions of Dopamine (3 Hydroxy-tyramine)	Dr. Albert Sjoerdsma Dr. David Horwitz	NHI
*Development of Self-Images and Self-Ideals in Normal Adolescence	Dr. Morris Rosenberg Dr. William Pollin	NIMH
*Proposal for the Use of Normal Controls and/or Employees of the Clinical Center in a Study of Antibody Levels Following Immunizations	Dr. Emil Frei III Dr. Richard K. Shaw Dr. Dane R. Boggs Dr. John P. Utz Dr. John L. Fahey	NCI
*To Further Define the Alterations in the Mechanical Properties of the Lung in the Normal, Cardiac, Bronchitic and Emphysematous Subject	Dr. Donald P. Schilder Dr. Dali J. Patel Dr. Donald L. Fry	NHI
*Use of Normal Controls in Blood Volume Studies, Amendment to Project No. H-40301	Dr. Frederic C. Bartter Dr. J. Picton Thomas Dr. Philip S. Chen, Jr.	NHI
*Studies of the Usefulness of Manifest Dreams Content for the Assessment of Covert Affective Reactions	Dr. Frederick Snyder Dr. David A. Hamburg Dr. Ralph W. Wadeson Dr. John Mason Dr. Edward Evarts	NIMH
*Approval to Use an Electrical Impedance Plethysmograph on Normal Volunteers, as Part of an Ongoing Research Project Entitled "Studies of the Interrelationships of the Nervous and Circulatory Systems. NIMH-CS-OC-(C)-5 (formerly NIMH 550)"	Dr. P. V. Cardon, Jr.	NIMH
*Addition to an Ongoing Clinical Research Project Entitled "Studies of the Interrelationship of the Nervous and Circulatory Systems. NIMH-CS-OC-(C)-5 (formerly NIMH 550)"	Dr. P. V. Cardon, Jr. Dr. A. F. Mirsky	NIMH
*Supplement to Approved Research Project, "Normal Perceptual Responses in Size Constancy, Apparent Movement, and Visual Illusion Situations"	Dr. V. R. Carlson Dr. Philippe V. Cardon, Jr.	NIMH

***Indicates projects utilizing normal controls.**

*Title	Investigators	Institute
*Request for Approval of Various Psychological Procedures with Normal Volunteers	Dr. V. R. Carlson Dr. D. Rosenthal	NIMH
*Proposal for Use of B-H^3 - and C^{14} -3-Methoxy-4-Hydroxymandelic Acid in Normal and Schizophrenic Man for Research Purposes	Dr. Seymour S. Kety	NIMH
*Reaction Time of Schizophrenics, No. M-P-C-(C)-15	Dr. David Rosenthal Dr. Theodore P. Zahn, Dr. William G. Lawlor Dr. David Shakow Dr. Philippe V. Cardon	NIMH
*The Self-Concept and Body Image as Related to Disease Susceptibility and Organ Choice	Dr. Isabelle V. Kendig	NIMH
*Studies on Adrenal Production Rate of Cortisol, Corticosterone and Aldosterone	Dr. F. C. Bartter Dr. P. S. Chen	NHI
*A Comparison of the Psychological Effects of Perphenazine and Chlorpromazine in Normal Control Volunteers	Dr. Conan Kornetsky Dr. Allan Mirsky Dr. William F. Weiss	NIMH
*Effects of Lysergic Acid Diethylamide (LSD-25) on Visual Functions, M-P L-1	Dr. V. R. Carlson Dr. D. Rosenthal Dr. T. Zahn Dr. Philippe V. Cardon, Jr.	NIMH
*Proposal for Study of Parkinsonism Produced by Ataraxic Drugs	Dr. N. Myrianthopoulos	NINDB
*Effects of Epinephrine on Lipid Metabolism in Man	Dr. Daniel Steinberg	NHI
*Effects of Social Pressures in the Ward Environment	Dr. Maitland Baldwin	NINDB
*Role of the Central Nervous System in Fat Metabolism (H-CS-OC(C)-5)	Dr. P. V. Cardon, Jr. Dr. P. S. Mueller (NCI)	NIMH
*Interrelationships of the Nervous and Circulatory Systems. (Effects of Chlorpromazine on Vascular Responses)	Dr. P. V. Cardon, Jr. Dr. Allan F. Mirsky	NIMH
*Request for Change of Principal Investigator on Previously Approved Project, "The Use of Films in the Study of Affects"	Dr. David A. Hamburg Dr. Ralph W. Wadeson, Jr. Dr. John Mason Dr. Joseph Handlon Dr. Morris Rosenberg	NIMH

Title	Investigators	Institute
Study the Effect of Morphine on Central Blood Volume in Normal Subjects	Dr. Eugene Braunwald Dr. Robert L. Frye	NHI
Relation of Emotional Behavior and Adrenal Function	Dr. David A. Hamburg Dr. Ralph W. Wadeson Dr. John Mason Dr. Joseph Handlon Dr. Frederick Snyder	NIMH
Fat-Mobilizing Activity of Urine from Fasting Patients	Dr. J. E. Rall	NIAMD
A Comparison of the Psychological Effects of Perphenazine and Chlorpromazine in Normal Control Volunteers	Dr. Allan F. Mirsky Dr. Philippe V. Cardon, Jr.	NIMH
Protein-losing Gastroenteropathy	Dr. Robert S. Gordon, Jr.	NHI
The Exercise Response in Normal and Cardiopulmonary Disease Patients: Cardiac Index, Stroke Volume Index and Oxygen Consumption Index	Dr. Samuel M. Fox III Dr. G. Octo Barnett	NHI
Studies on Carbohydrate Metabolism	Dr. James B. Field Dr. Arnold Weinberg	NIAMD
The Metabolism of C^{14} Labeled Galactose in Man	Dr. Stanton Segal Dr. Yale J. Topper Dr. Alberta Blair	NIAMD
Amendment to Approved Request of October 31, 1957, entitled "Permission to Collect Expired Air from Normal Controls"	Dr. Donald M. Watkin	NCI
Request for Permission to Determine Cardiac Output by the Indicator-Dilution Technique in Normal Subjects Before and After Receiving the Pressor Amine, Dopamine	Dr. David Horwitz Dr. Leon I. Goldberg	NHI
Trial of MER-29 in a Normal Control with Hypercholesterolemia	Dr. Daniel Steinberg	NHI
The Measurement of Visual Pigments in Man	Dr. W. A. H. Rushton	NINDB
The Relationship of Pre-School Behavior to Retrospective Reports of Neonatal Behavior (This project utilizes "subjects" at Halfway House)	Dr. Richard Q. Bell Dr. D. Wells Goodrich	NIMH

Title	Investigators	Institute
Permission to Administer "Ateroid", an Alkaline Extract of Hog Duodenal Mucosa, to Normal Control Subjects	Dr. Donald S. Fredrickson Dr. Joseph H. Bragdon	NHI
Antagonism between Triiodothyronine and Reserpine with respect to Cardiovascular Effects and Effects on Lipid Metabolism	Dr. Daniel Steinberg Dr. Eugene Braunwald Dr. Robert L. Frye	NHI
To Evaluate in Normal Volunteers Several Intestinal Absorption Tests Currently in Routine Clinical Use (A8-801)	Dr. Leonard Laster Dr. John W. Singleton	NIAMD
Request for Permission to Administer Low Concentrations of Norepinephrine to Normal Controls by Intravenous Infusion over an Eight Hour Period	Dr. David Horwitz Dr. Eugene Feigelson Dr. Daniel Steinberg	NHI
Effects of Taurine on Cholesterol Levels in Man	Dr. Daniel Steinberg Dr. Joel Avigan	NHI

Calendar Year 1959

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY REPORT

CLINICAL CENTER

CLINICAL AND PROFESSIONAL EDUCATION

CC-02
Serial No.

This organization administers and services a variety of programs of professional education and other activities with educational implications for the Clinical Center and the NIH.

Report on Major Program Activities:

These activities are not only of administrative interest but of concern to a number of diverse program areas. For this reason, and to afford a better opportunity for their review, individual and somewhat more detailed reports have been prepared for each program. These are attached as a portion of this report. A few brief highlights are reported below:

Clinical Associate and Research Associate "Matching Program":

This is a new program that has evolved to meet the problems created by the growing interest of young physicians in the programs of clinical research and basic research education at NIH. The large number of applicants and the fact that most applicants desired to be considered by several Institutes, and for both basic laboratory and clinical research, made it necessary to develop some central mechanism for handling applications. The plan that was developed was designed to conserve the time of our senior scientific staff and to meet administrative needs without interfering with flexibility and autonomy in Institute educational programs.

The "matching" feature is similar to the National Intern Matching Program. It resolves the problem of final selection of candidates by matching the candidates' desires with those of the Institutes on the basis of rank order lists submitted by both parties. A revised program for 1960 operation has been approved.

Some statistics of interest: Over 1,000 inquiries were received. More than 346 applications were requested. 213 applications were completed and considered. More than 625 interviews were granted and arranged. Six Institutes participated. 5,000 copies of the brochure "Building Medical Research Manpower" were circulated. These figures are more remarkable when one takes into account the very careful effort that was made to discourage applicants whose background did not meet our standards, or whose interests and training had not prepared them for research. Some of the latter were recruited for other PHS programs.

Clinical Fellows (Residents):

The professional service departments of the Clinical Center selected their residents through a separate mechanism, as did the NINDB. The following appointments were made:

Clinical Pathology - 7
Radiology - 3
Neurology - 3
Pathologic Anatomy - 6
Oral Pathology - 1

All other residents were selected through the "Matching Program" and then nominated by residency appointment in the usual manner.

Internal Medicine - 6
Psychiatry - 7

Research Associates:

Three years ago the Scientific Advisory Committee for the NIH programs of the USDA Graduate School developed a program of training for young physicians designed to prepare them for the laboratory sciences. Under this program, Institutes appointed outstanding physicians completing their internship or one year of residency to positions in their laboratories. The laboratory chiefs assumed responsibility for training as preceptors; the Scientific Advisory Committee organized and provided instructors for a formal program of instruction in the sciences. This office administered and serviced the program. There are seven second year men and ten first year men in the program in 1959. Schedules and arrangements for 171 hours of instruction for the first year men were made by this office. Weekly agendas and special bibliographies for seminars were prepared for the second year men. Weekly Journal Club notices were prepared for the entire group.

Activities in Support of Clinical Education in the Clinical Center:

This office provided a number of staff services to the Education Committee of the Medical Board of which its Chief is Chairman. This committee conducted 13 Combined Clinical Staff Meetings, Quarterly Clinical Staff Meetings and Clinicopathologic Conferences. A list of these programs which are designed to provide inter-disciplinary and inter-institute exchange of professional knowledge follows:

Date	Kind	Topic	Attendance
1/8/59	QCSM	Discussion of Malpractice Liability	128
1/22/59	CCSM	Idiopathic Hypoalbuminemias	88
2/12/59	CCSM	Studies in Diabetes Mellitus	115
3/26/59	CCSM	Primary Amyloidosis	99
4/9/59	CCSM	Porphyria	127
4/23/59	CCSM	A Neuroendocrine Approach to the Investigation of Emotional Processes Human Behavior and Adrenocortical Function	93

Date			
5/28/59	CCSM	Peridontal Disease	65
6/11/59	QCSM	Radiation Safety Practices at NIH	72
9/24/59	CPC	Problem in Differential Diagnosis	95
10/8/59	CCSM	Hypothermia in Neurological Investigation and Treatment	75
10/22/59	CPC	Pneumoconiosis	62
11/12/59	CCSM	The Reaction of Serum from Patients with Systemic Lupus Erythematosus with Nucleoprotein and DNA	128
12/10/59	CCSM	Some of the Causes of Cancer in Man and Mouse	151

Late in the year, after a study by a task force of the Medical Board, the committee revised the staff meeting pattern to provide one meeting a month and discontinue the formal Quarterly Clinical Staff Meeting with the understanding that any meeting might be used to present administrative information to the professional staff.

During the year the committee reviewed and approved the applications for the conduct of Residency Programs in Dermatology and Peridonia. It also reviewed the Radiology Residency Program and approved its extension from three to four years to permit more instruction in the field of radioactiveisotopes. The committee's recommendations were approved by the Medical Board and by the Director of NIH. The programs are now under review by the appropriate American Specialty Boards. Approval will bring the number of approved Residency Programs from eight to ten.

The Chief of Clinical and Professional Education attended the Congress on Medical Education and Licensure sponsored by the American Medical Association and the Meeting of the Association of American Medical Colleges for the purpose of stimulating applications for training at the Clinical Center and to interpret our programs to Deans and other Medical School officials. This also served to keep him in close touch with recent developments in Graduate Medical Education.

In 1958 the NIH encountered difficulty in securing training and practice credit from the American Board of Internal Medicine for its Clinical Associates and Residents. Very active representation to that Board by this office and members of the Medical Board, together with a joint rebuttal of the American Board's statement about Defense Department policies by NIH and the Surgeons General of the three Armed Services brought about some softening of this certifying organization's rulings. The current position of the American Board of Internal Medicine is that any person engaged in clinical work in Internal Medicine at the Clinical Center will receive practice credit for the time he spends in that work without regard to previous fulfillment of training credit requirements. We are continuing our efforts to obtain recognition of education received on our medical services for purpose of training credit requirements.

The Education Committee met 11 times during the year to recommend residents for appointment, plan staff meetings and to select four meetings which should be submitted to the Annals of Internal Medicine for publication. A very active subcommittee made the primary selections for publication and supervised the editorial work on these manuscripts. This represented a major contribution of time by the Chairman, Dr. C. Gordon Zubrod.

Staff support for all staff meetings was supplied by this office.

Commissioned Officer Student Training and Extern Program (COSTEP):

The COSTEP Program entered its fourth year of formal organization in 1959. This program is designed to introduce young professional students to the PHS at an advanced stage in their predoctoral training through providing them with employment affording an opportunity for professional growth. The Chief of Clinical and Professional Education has served as Chairman of the PHS COSTEP Committee since its organization in 1956-57.

In the summer of 1959 seven Institutes and two Divisions employed 32 medical, two nurse, two scientist, one veterinarian, and three engineering students. COSTEP is a year-round program and a small number of students have been employed during periods other than the summer months since 1957. The medical students this year were drawn from 20 U.S. and one Canadian School of Medicine.

Evaluation schedules completed by students and preceptors and an analysis of other program records has yielded information of specific interest.

a. In 1957 forty-five per cent of the medical students came from schools located in Virginia, Maryland, and the District of Columbia; in 1959 only twenty-five per cent of the medical students came from these schools.

b. Sixteen COSTEP officers have applied for Clinical or Research Associate positions - six have been appointed, two advised to apply for 1962, one accepted a position with CDC, two under consideration, and five rejected. 1/

c. Forty-six per cent of the medical students indicated an interest in a PHS internship. 2/

1/ Based on students participating in the program 1955 through 1959.
2/ Based on 30 completed questionnaires and 28 preceptor questionnaires.

d. Eighty-seven per cent of the students expressed a desire for active duty following graduation. 2/

e. Ninety per cent of the students indicated they had other opportunities for professional appointment. 2/

f. Seventy-five per cent of the preceptors reporting felt their students (a total of 21) worthy of a special recruitment effort. 2/

g. The first woman veterinarian to be appointed to the PHS Commissioned Corps (active duty DRS since July 1959) came into the Service via NIH COSTEP duty.

In April 1959, at the request of the Community Services Branch of NIMH, the possibility of assigning COSTEP students to "training duty" positions in State Mental Hospitals was discussed with PHS-Division of Personnel and the Chief, Clinical and Professional Education in his capacity as Chairman of the COSTEP Committee. Guidelines were successfully worked out for NIMH. In December 1959, NIMH advised this office that the Regional Medical Director, Region V, wished to have four COSTEP students to participate in a program to be carried out jointly by the Regional Office and the State of Wisconsin. This is the first time a request of this type has been made for COSTEP students.

The program continues to have the problems of limited space (many scientists indicate they would like to have a COSTEP student but have no physical space in which the man could work) and budget. On the latter item, COSTEP positions, unfortunately, are usually in direct competition with regular positions. However, it is encouraging to note that estimates of positions for 1960 are running slightly ahead of those for 1959.

Training Outside the Service:

There has been a significant increase in the number of persons applying for training outside the PHS from NIH during 1959. NIH employees are trained under two separate authorities: Commissioned personnel under the PHS Act; and Civil Service personnel under Public Law 85-507.

Passage of Public Law 85-507 in 1959 gave a new impetus to the recognition of needs and demands for training at NIH. The training of our staff both within and without the Federal government is becoming a major activity and almost a program within itself. The growth of this

2/ Based on 30 completed student questionnaries and 28 preceptor questionnaires.

activity with its financial implication and its impace upon research programs is forcing a review of policy and the development of mechanisms for evaluation of justification for training and benefits from training. Within the legal framework of PL 85-507 and the PHS Act, NIH expects to develop sound educational methods for improving the effectiveness and efficiency of its staff. As a result of the passage of PL 85-507 this office was given the added responsibility of reviewing all applications for training of Civil Service personnel as well as for Commissioned personnel. Training given NIH personnel during 1959 almost doubled that of the previous year. Some statistics of interest are:

a. Applications reviewed by this office: 126 employees were approved for training at facilities outside the Federal government.

b. Training arranged by this office: 319 employees were given training at other government agencies.

c. Training provided by this office: 1,295 employees were given inservice training on an orientation basis.

d. Training arranged by Institutes and Divisions: 504 employees were given inservice training of a professional, scientific or technical level; 50 employees were given inservice training in management and supervision; 410 employees were given inservice training of a clerical nature.

NIH-USDA Graduate School Program:

The program of night courses conducted by NIH-USDA Graduate School Program, continued to be of great interest to our employees and those of nearby agencies. In the spring semester 241 persons registered for the 17 courses that were given; in the fall semester 429 persons registered for the 21 courses that were given.

It is our judgment that the registration figures do not indicate the full extent of the demand for this type of education. Inquiries received by this office indicate that a broader curriculum would attract an even larger number of students.

The trend toward scientific courses noted in previous reports continued in 1959. Interest in courses in administration and the liberal arts continued to decrease.

During 1959 the Scientific Advisory Committee stimulated the organization of a nonprofit corporation called "The Foundation for Advanced Education in the Sciences, Inc.". They have formulated a proposal for consideration by NIH and the Surgeon General which provides for the operation of this program by the Foundation and discontinuance of the present arrangement with the Graduate School in order to obtain a more flexible and effective administration for the night school program. This proposal is under study at the present time.

NIH Television Unit:

A full report of the activities of this unit, outlining its administration through the Clinical Center, the history of its development and the work performed during 1959 has been prepared. Some of the highlights of that report follow.

a. WNIH-TV: The Clinical Center system produced 18 programs which were viewed by 3,430 people during 1959. Approximately 2,225 visitors were given a first hand view of NIH at work with a very great saving of professional time by this method. Twelve of the programs were for professional groups. A number of novel techniques were employed which demonstrated the methodology's usefulness in extending the scientist's and physician's ability to communicate successfully with his colleagues.

b. Research: Five scientific papers resulted from the research conducted in 1959. A sixth paper is in preparation for presentation in April. The volume of research work was adversely affected by the shortage of personnel.

c. Planning and Consultation: In addition to consulting with scientists regarding such problems as automation of the counting of tissue culture cells in mitosis, the planning function has been concerned with defining the needs of the Clinical Center system and the New Surgical Wing. The latter has consumed considerable time as the design of the operating rooms presented unusual optical problems. The project report includes the results of these studies and estimates of the costs of meeting the needs.

The report discusses the problems encountered during the year with special reference to the difficulties in securing personnel and budget. It concludes with the recommendation that authority to granted to hire one additional technician in each quarter of calendar year 1960 in order to bring the project to its minimum adequate personnel strength.

The budgetary impact of this recommendation is reviewed and sources for obtaining the modest sums required are suggested. It is pointed out that NIH leadership in this field can not be maintained without increased support for the project.

Orientation Programs:

NIH Orientation (Compass) Course: The revision of the course which took place in mid 1958, on an experimental basis, has been well received. The major initial change was from a pattern of one hour presentations on five separate days to a one-half day program. The first one-half day program took place on June 26, 1958. Changes in content and techniques have been made based on observation and study. A specific example: Employee health services are now presented via a dramatic skit, and television (WNIH-TV) the Clinical Center system, which began in late 1958, has provided ~~the major~~ favorable impact upon the course. New NIH employees are now able to see research in action - including laboratory and clinical phases. In October and December 1959 the NIH Orientation Course and the NIH segment of the PHS Management-Orientation Course were coordinated so that the examples of "research in action" picked up by television could be televised simultaneously to the 14th floor Assembly Hall (55 drawn from all Bureaus of PHS) and the Clinical Center Auditorium (150-300 new NIH employees). Prior to this time TV had been used independently for each group. The coordination, obviously, made possible a much greater return with a subsequent decrease on the demands of scientific and professional personnel. Kinescopes have been made of some of these programs and other programs similar in nature. These kinescopes have already proved very useful in interpreting NIH to visiting professional and non-professional groups.

In 1959 five courses were attended by a total of 989 NIH employees. A Question & Answer Period for Commissioned Officers (primarily for new C.O.'s, but also open to veteran officers) was held as an integral part of each course. Serving as resource people for these meetings were representatives from OSG-PHS Personnel, NIH C.O. Unit, and the Clinical and Professional Education Branch-CC. Eighty-six officers attended these sessions. A number of administrative personnel interested in rules and regulations pertaining to the CORPS have also attended.

PHS Management-Orientation Course: The NIH segment (one day of a five day program for C.O.'s and GS-9 and above nominated by their respective Bureaus) was presented on the following dates: December 3, 1958; May 12, 1959; October 6, 1959; December 15, 1959. Approximately 55 persons attended each program.

Special Orientation Programs:

During the year seven programs were held. These are listed below:

Date	Program	Attendance
3/25/59	Meeting for C.O.'s Planning to Inactivate in July	100
6/22/59	High School Science Teachers	19
7/9/59	Commissioned Officers Reporting to NIH (Primarily for phyicians with patient care responsibilities)	93
7/14/59	COSTEP and Civil Service Summer Employees	100
7/24/59	COSTEP-Visit to PHS Hospital - Baltimore	13
7/31/59	High School Science Teachers Attending Summer Institutes Conducted by Area Universities	370
9/11/59	Program for Medical Students (Planned and coordinated with Special Events)	150

PHS Orientation for New Employees: Approximately one-half day of this two-day program is spent at NIH. Following a general orientation and tour of the Clinical Center, conducted by the Special Events Section, the group is addressed by the Chief of Clinical and Professional Education regarding the overall mission of this Bureau. Five such sessions were held in 1959; the average attendance for each session was 37.

High School Science Teachers Chemistry-Physics Institute:

This eight week program for high school teachers of science sponsored by American University through a grant made by the National Science Foundation was conducted for the fourth year in 1959. Nineteen teachers from ten states and the District of Columbia reported to NIH on June 22. The following NIH Institutes and Divisions participated in the program: NCI, NHI, NIAID, NIAMD, NIMH, CC, DBS, DRS. The teachers worked in NIH laboratories (on a non-pay basis) from 10:00 a.m. to 5:00 p.m. every Wednesday, Thursday and Friday.

Evaluation of the program was very favorable. Some specific items of interest lifted from "reaction sheets" completed by 15 preceptors are as follows:

a. Teacher's performance and relationship to estimate of background

Above average - 6 Average - 9

b. Teacher's contribution to work of laboratory

Great deal - 2 Some - 10 Little - 3

c. Teacher's growth in and adjustment to work in laboratory

Excellent - 9 Good - 6

d. Fourteen of the 15 preceptors reporting indicated that they would welcome a continued program with teachers next summer.

Dr. Leo Schubert of American University, Co-Director of the Teacher Institute, reports that the teachers' evaluation of their experience at NIH was complimentary in all respects.

A final item of interest is the fact that one of the teachers who participated in the 1958 Institute later received an American Association for Advancement of Science Fellowship and returned to NIH in 1959 to continue work on "Obesity" (his original 1958 project).

Counseling and Consultation:

Student counseling - ranging from precollege level to graduate level - estimated per annum: Interviews - 100; telephone and written inquiries - 75. Counseling professional staff regarding their educational problems averages 50 individuals each year. This office is also consulted on a variety of educational activities ranging from problems in establishing educational programs in other agencies to preparation of educational materials for NIH use or by other Bureaus of the PHS. We also evaluated audio-visual materials used in PHS recruiting by using them with test audiences.

In-Service Staff Training:

During 1959 arrangements were made for Mr. Staton to have several exposures to the medical school environment. To date he has visited Georgetown Medical School on numerous occasions, attending selected classes and laboratories and discussing various phases of medical education with professors and students. One similar type experience was obtained at the University of North Carolina Medical School in conjunction with his attending a conference on public health education conducted by the University School of Public Health. He also attended the annual meeting of the American Association of Medical Colleges, which was held in Chicago, and the Medical Education for National Defense meeting, which immediately preceded the Chicago meeting. This experience has proved valuable to Mr. Staton in discharging his responsibilities in this office which call for his working with physicians and scientists and for counseling of young professional students. It is planned to continue this in-service training in 1960.

PHS Committees:

The Chief continued to serve on the Committee on Residencies and Internships; the Committee on Training Outside the Service; the Committee on the Commissioned Reserve; the CORD Committee and also served as Chairman of the Committee on COSTEP. He was alternate for the PHS representative on the Inter-Service Committee for the MEND Program. This has involved attendance at 19 committee meetings during the year.

Miscellaneous:

The office prepared the overall report of training activities for all of NIH for fiscal year 1959.

A questionnaire study of the need for bi-weekly resident's clinical rounds was conducted and this has resulted in the re-establishment of these essentially junior house officer operated conferences.

Inspection of our residency training programs by accrediting bodies was arranged and conducted on three occasions during 1959.

The annual report on Residency Training was prepared for the American Medical Association. The Interns and Residents Report was also prepared for the AMA.

The data on NIH residency programs was prepared for inclusion in the PHS announcement on clinical training.

Plans for reorganization of the work of the office was prepared and put into effect. Adjustments in the working responsibilities of members of the staff has been partially accomplished. Final adjustments, which are dependent upon action on the new proposal for the conduct of the NIH Graduate School Program, have been deferred until an administrative decision is received.

During the year the Chief was made a member of the Executive Committee of the Microcirculatory Conference, Inc., and was Chairman and a member of the Executive Committee of the Council on Medical Television of the Institute for the Advancement of Medical Communication.

Attachment I

Serial No. CC-0

ANNUAL REPORT

Television Project - National Institutes of Health

The television activities of the National Institutes of Health are administered through the Clinical and Professional Education Branch of the Clinical Center. The scope of the activity is quite broad, embracing the exploration of the value of television and related electronic techniques in our research environment as well as the study of its application to education and communication, and the operation of what has now become "WNIH-TV - The Clinical Center System".

The project came into being after a series of studies in 1955 revealed a real potential for this medium as a tool in patient care, scientific education and communication, and biologic research. This series of practical tests was kinescoped and reported in a film containing excerpted specimens of the result. The film was titled "Electronic Image Processing", a term coined by our group and now found in the scientific literature as the generic for the use of scanning techniques in biologic research. In turn, this study developed from four years of intensive work on the part of Mr. Donald Snow (then in Research Facilities Planning) and the Chief of Clinical and Professional Education. This work was directed toward the design of the uniquely flexible television distribution system of the Clinical Center.

The period 1956 through 1958 was devoted to installation of the distribution system, planning and equipping the central control room, and acquiring equipment. A remarkable variety of electronic gear was donated by industry and Federal agencies which permitted intensive research and a beginning of the use of television for communication purposes in education. During fiscal 1957 and 1958 funds were provided by several Institutes as direct expenditure items at the discretion of the project. In fiscal 1959 a smaller sum, $16,000, was made available from Director's reserves through the Clinical Center for the specific purpose of supporting the research activities of the project. In July 1959, a similar amount was made available, accompanied by a directive to focus activities on education and communication during fiscal 1960. The Clinical Center included an item of $30,000 in its fiscal 1961 budget request for continuation of the latter aspects of the project.

CALENDAR 1959 ACTIVITIES

General

During 1959 progress was made in research, program presentation and planning for the incorporation of TV facilities into new NIH construction. The period was characterized by an increasing demand for television services by researchers and administrators; demands that far exceed our

resources. It was also marked by our increasing potential for service, much of which could not be realized because the manpower permitted by the modest budget was not equal to our other resources. This increasing potential resulted from our acquisition of additional equipment in July, and from a variety of new and valuable applications perceived by NIH staff members and by our group.

Despite the limitations that have been mentioned it was a year of real progress and productivity.

WNIH-TV: The Clinical Center System

It had been evident for some time that there were a number of problems at NIH that could be solved or alleviated by TV. It was also evident that conventional TV techniques were poorly adapted to NIH needs and that new methodology should be evolved. It was suspected that similar techniques would be a real contribution to the use of television in any educational situation.

By 1957, the visitor problem had become so acute that an Ad Hoc Policy Committee was assembled to devise methods of conserving the time of our scientists that was being devoted to individual and group visitors. Two conclusions resulted: visitors should be consolidated into large groups whenever possible; and, organized presentations should be made to these groups, with television substituting for the laboratory tours.

In the meantime, we were aware that TV could be of value in increasing the effectiveness of scientific presentations at medical and scientific meetings.

We also had evidence that TV could be used to care for overflow audiences by carrying the presentation in one auditorium to groups gathered in other meeting places.

By late 1958 we were in a position to begin tests of our concepts. We had a well developed philosophy about the use of the medium based upon the employment of equipment that took the studio to the locus of activity. Such equipment must be designed to be unobtrusive and undisturbing to the environment. By this time we had assembled minimal equipment suitable for the purpose which required no bright lights, occupied little space and could be conveniently transported.

Beginning on December 3, 1958, with only four earlier productions as experience, we began to employ television for these purposes.

In the past twelve months 18 programs utilizing TV have been presented to audiences totalling 3,430 persons.

Approximately 2,225 visitors have been given a first hand view of NIH at work. On a single day, July 31, 370 high school science teachers were gathered in the Clinical Center auditorium. They were toured, via TV, through the Laboratory of Chemical Pharmacology of the Cancer Institute and visited the General Medicine Branch's Leukemia Nursing Unit. Of the 188 questionnaires returned, 183 rated the program good to excellent; 152 stated that the part they liked most about the program was the closed circuit TV. Such comments as "I have visited NIH before and found today's TV technique much superior to individual lab visitations" and "The naturalness of the whole thing and the ease of viewing without crowding into wards" were typical. More important, Special Events, whose cooperation has been outstanding, made an analysis of the relative time involved in this method of presentation vs. the conventional method of handling visitors. They estimate that this one group would have required three full weeks of scientist time if they had visited in the usual manner and that the same amount of their staff's time would have been involved!

Several unique techniques were employed during the 12 programs for professional groups. A new method of handing the scientific exhibits at a professional meeting was evolved for the Seventh Microcirculatory Conference in May and the Second Pan-American Congress on Rheumatic Diseases featured a live clinic which was televised from the stage to a large screen in front of the stage which made it possible for the audience to see every detail of the living pathology presented. Dr. Daft later reported to the Director of NIH that this was a "striking demonstration of the informational and educational value of television".

This portion of our activities is briefly enumerated in an annotated appendix to this report. It will be noted that the programs became increasingly complex as our skills improved and with the addition of new equipment (1 camera and a kinescope recorder) early in the fiscal year.

During 1959 the NIH group transmitted one program via micro-wave from HEW to NIH. The Russian visit was transmitted to WRAMC and they transmitted color programs in Surgery to NIH. There have been a number of inquiries from our professional groups regarding the possibility of transmitting NIH program material to national meetings in downtown Washington in 1960. Some very early planning is in progress.

The OSG, PHS is currently studying the costs of visits of its "high level" orientation groups to NIH versus the costs of presenting the NIH material via micro-wave from NIH to DHEW. These may prove quite favorable toward micro-wave if the service can be shared by other groups that currently must travel between these points to conduct their activities.

Inter-Institutional Communication Via Television

NIH suggested the usefulness of TV communication between Walter Reed, NIH and National Naval Medical Center in 1955. As a result of experiments conducted by NIH and Walter Reed employing micro-wave in 1956-57 a network has been established which serves a number of medical installations in the metropolitan and nearby areas. NIH is not currently participating. During 1959 other participants have continued to urge that we join in this activity. Three methods are potentially available:

1. Micro-wave: This service costs about $13,000 a year if furnished by the telephone company or a capital cost of $28,000 plus several thousand dollars a year in maintenance if operated privately. It provides only point to point communication.

2. Low Power Broadcasting: If frequencies in the 420 mc band are available in response to our recent request directed to the Inter-Agency Committee on Radio Frequency Allocation there is a distinct possibility that communication from NIH to multiple points can be achieved at costs measured in hundreds rather than thousands of dollars.

3. Cable Communication with NNMC: This can be achieved for about $3,000 capital cost and practically no recurring expense. It will permit transmission to WRAMC via Navy's micro-wave and reception from WRAMC of such programs as Navy may select.

The Low Power Broadcast alternative is most appealing as it may serve many purposes due to its ability to reach a variety of points. Local problems of communication between buildings and such administrative problems as communication between Silver Spring and NIH for the Cancer Chemotherapy Center (structural formulae) could be solved.

Kinescope Recordings

The recording of television programs on 16 mm. motion picture film is a method of preserving this information for future use that has many advantages in our situation. It is preferable to tape recording as it permits one to re-use the material wherever a motion picture projector is available. We had predicted that this would save time of our scientists by allowing us to re-use presentations made for one set of visitors with subsequent groups. It would also find use as a means of making research records in the behavioral sciences. A variety of other uses can be readily suggested.

In July the unit acquired a black and white film recorder. The equipment we acquired is a motion picture camera with a special shutter and a monitor with a special picture tube. It is not the ideal gear for the purpose, but is the only available alternative to an instrument costing $24,000 which we felt was too expensive to be considered. A number of recordings have been made. We are still dissatisfied with the quality of our recordings. "Shutter bar" is always difficult to eliminate in a system like ours. This has been overcome. We are still having difficulties in standardizing exposure and processing, and cannot obtain

a good gray-scale in our pictures. These difficulties are a recognized part of any new kinescope installation and can be solved by hard work over an extended period and good cooperation by the commercial processors. Our shortage of staff time has been the factor responsible for delay in accomplishing this.

Despite these difficulties the film recordings we have produced have been quite useful. Some of the films we have made and their subsequent uses are indicated below:

Color Kinescope (for research purposes) made of Dr. Maitland Baldwin's (Chief of Surgical Neurology Branch, NINDB) work with rhesus monkeys and effects of radio waves.

October 16, 1959 - MEND Symposium for Professors of Preventive Medicine and Council on Medical Television.

Presented live by Dr. Baldwin.

Audience - 150

Color Kinescope - "Physiology and Pharmacology of the Retina"

November 20, 1959 - Society of Professional Editors

Presented by Dr. Murray C. Brown

Audience - 25

Operation "K" - Visit of USSR Minister of Health (Dr. Markov)

A complete black and white kinescope (2-3 hours coverage) was made of this event which took place on September 25, 1959.

"Germ Free Laboratory" - Dr. W. Newton of NIAID and Mr. John L.S. Hickey of DRS

October 16, 1959 - MEND Symposium for Professors of Preventive Medicine and Council on Medical Television.

Presented live by Dr. Newton

Audience - 150

November 20, 1959 - Society of Professional Editors

Presented live by Dr. Murray C. Brown

Audience - 25

December 16, 1959 - Gonzaga High School

Presented live by Mr. Hickey.
Program arrangements by Special Events.
Audience - 180

"Background of Operation K and Section on Gout" - Dr. Bunim of NIAMD

October 17, 1959 - High School Science Teachers
(NIAMD and NCI sponsored)

Audience - 225

PHS Management-Orientation Conference and NIH Orientation -

December 15, 1959 - "Hematology Section of Clinical Pathology Department of Clinical Center" - Dr. Russell Little and staff

"Section on Blood Bank - LBBP-DBS" - Dr. Paul Schmidt and staff

The black and white equipment has not yet been used in research due to lack of staff time. Dr. Baldwin has a specific clinical project to be done as soon as staff time can be made available. Our use of color kinescopes in research is reported elsewhere.

RESEARCH - APPLICATIONS OF TELEVISION TO BIOLOGICAL PROBLEMS

In 1955 we coined the term Electronic Image Processing, which is now a generic in the literature, to describe the use of television and related electronic techniques for obtaining quantitative information from any subject matter that could be visualized. During 1959 we continued our work in this field.

Considerable work was accomplished earlier in the year and a number of papers were completed based on earlier work and on work done in that period. The increasing demands of the TV activity in educational and informational areas, the extremely limited personnel available and the fact that the funds made available in fiscal 1960 were directed toward program TV seriously hindered research activity in the latter half of the year. There were some definite accomplishments despite these limitations.

Papers presented as written or film reports during 1959 are listed below:

Electronic Image Processing. Mark III. Circulation in the retina and pia. Brown, M.C.; Laskowski, E.J.; Warner, L.; Potts, A.M.; and Whitehouse, W. C. Microcirculatory Conference - October 1, 1958. (Summary in press). Revised and extended January 1959. Presented at the Institute of Physiology, Lund, Sweden, January 1, 1959 (by invitation).

Electronic Image Processing. Mark IV. Observations of hemodynamic and pharmacologic phenomena in living retina. Brown, M.C.; Laskowski, E.J.; Whitehouse, W.C.; Potts, A.M.; (introduced by Warner, L.) Presented at American Association of Anatomists - Seattle, Washington, April 2, 1959, Anat. Rec. 133: 461, February, 1959.

The Effects of Certain Radio Frequency Energy on the Monkey. Baldwin, M.; Bach, S.A.; Lewis, S.A.; Brown, M.C.; Whitehouse, W.C.; Presented at the American Psychiatry Association, Philadelphia, April 28, 1959. Paper accompanied by film (in press).

Unilateral Intermittent Spontaneous Dilatation of Retinal Veins of Undetermined Etiology Associated with Blindness. Longfellow, D.; Brown, M.C.; Presented at the Academy of Ophthamology Meeting, October, 1959 (by invitation) (in press).

Television and the Future of Undergraduate and Postgraduate Education. Brown, M.C.; Presented at the American Medical Writers Association, New York Chapter, Rockefeller Institute, New York, April 30, 1959. (in press).

Late in the year the parameters involved in converting the color television ophthalmoscope into an instrument that was capable of more quantitative measurement of biochemical and pharmacological phenomena were successfully studied. This study was paralleled by tests of the feasibility of the use of flying spot scanning techniques for similar studies. This resulted in a successful first approximation. The work in color indicates that our future film recordings should also have much better pictorial quality. The latter point is of limited scientific importance as most of our measurements are made by other means, but is of importance in teaching and the production of clinical records. In the course of this work we successfully tested the hypothesis that an inexpensive vidicon black and white ophthalmoscope could be produced. These developments all involve optical and lighting requirements that are somewhat sophisticated. Practical optics and light sources for routine application require considerable engineering beyond our primitive bread board layouts. Fortunately, Bausch and Lomb have become interested in the possibilities and are expected to provide consultation and engineering assistance in the immediate future.

Early in the fall a new system was devised for animating line drawings. It was conceived as a means of demonstrating the technique of line selection but has a variety of teaching and demonstrating applications. The system consists of an inexpensive flying spot scanner, a monitor and polaroid materials. A drawing is prepared on a transparent material. The drawing is then overlaid with polaroid materials cut out to follow its outlines and oriented in such a manner that parts that are to simulate motion are on an axis that is alternately crossed and parallel to the polarized axis, a round polaroid plate as it is rotated by a small motor. The transparency is placed in the light path of the scanner. The rotating plate is placed in the same plane between the scanner and the transparency. The assembled polaroid elements permit the transmission and occlusionof light in the selected parts of the drawing as a the polaroid plate is rotated by a small motor. The photo cell that is viewing the scanner tube through the drawing and the polaroid plate varies its response as the polarizing effect changes. The monitor changes its picture to correspond. We have not published this development because we have not yet investigated the complications that might ensue due to the fact the device has commercial value in the field of advertising.

We have been much more concerned about the work that has not been done. We have continued some study of pathology in human eyes with the Walter Reed group but this has been severely limited by available staff time. We also demonstrated the feasibility of studying the albino rabbit choroidal vessels with the color ophthalmoscope. We would like to confirm Leopold's studies of the pharmacology of these vessels by this method

as he employed a surgical approach to the vessels he studied while we can examine an undisturbed vascular bed. We would like to continue collaboration with Dr. Leon Jacobs by making further recordings of the natural history of toxoplasma in the rabbit retina. If possible, the feasibility of recording tagged fluorescent antibodies in these animals should be explored. These studies were discontinued last summer because of limited staff time. We would like to resume experiments designed to measure the effects of pressor amines on retinal vessels in humans. A first experiment recorded for Dr. David Horowitz suggested that a sensitive quantitative method could be developed if sufficient time could be devoted to the undertaking.

Other well defined studies are awaiting an opportunity for attention by the technical staff. Dr. Baldwin wishes to record circulatory changes in brain associated with seizure; he also has a clinical problem. The latter concerns fractionation of the automatism observed during seizures in an effort to more precisely assign the phenomena observed to the region of the brain involved. The equipment for remote observation of patients during periods of expected seizure, the means of immediate remote focussing of the viewing system on the affected body parts, and the means of recording the phenomena on motion picture film are available and awaiting use when staff can be freed to do the work.

Dr. Richard Bell's study of nursery school children has been equipped for remote viewing via television. This equipment awaits certain cables and connectors before it can be installed. The staff time requirements for placing it in operation are minimal and the system should be operative in January. Equipment with extremely simple operating controls was prescribed and the project should require very little support.

The purchase of new radiologic equipment for the cardiac catheterization laboratory incorporating TV provides an exciting opportunity to improve upon the currently available presentations of fluoroscopic images through processing techniques with which we are familiar but which are not currently in use.

Our interest in fluorescence continues but no progress was possible in 1959. Dr. Richard Malmgren's group in NCI have revived their interest in use of these techniques for automating the counting of cancer cells from human blood on micropore filters.

Some development work was done during the year. A "Crispener" was built for processing and improving the color signals used in research. A new and simplified "gray scale generator" was developed and built as a test instrument for use in the WNIH system for standardizing kinerecorder set up and quick checks on equipment performance. A variety of older equipment was reconditioned for test and operational use.

POSITION OF NIH IN THE FIELD OF MEDICAL TELEVISION

Despite our very modest investment in television, NIH has assumed a position of leadership in medical television. Our research activities have been recognized and we have made contributions to the use of television in medical education. We have provided support for the Council on Medical Television which met at NIH twice in 1959 and is scheduled to meet here in 1960. We have also contributed some leadership and stimulation to others through this organization. We have also had requests for consultation from medical schools.

We are concerned about our ability to maintain our position in the field. There is a sudden intensification of interest in medical television, particularly among medical educators. Medical schools are assembling resources for using the media in undergraduate education and active undergraduate and postgraduate programs are being developed. Television-like devices are becoming a part of medical care instrumentation and increasing numbers of investigators are using television tools. Many of the applications we have conceived are being made first by others. In the meantime the things we would like to explore and develop at first hand cannot be done well or at all because we lack the minimum technical staff for their accomplishment. This is a lack of staff time rather than skill.

It seems important to us that NIH maintain a position of knowledgeability and leadership in this field.

Problems

The most serious difficulty we have encountered is the shortage of technical personnel which is discussed below.

Another very serious problem has been our handling of sound for WNIH presentations. There are deficiencies in the sound systems in both Clinical Center auditoria which should be corrected. The present equipment is also poorly adapted for the uses in which we are interested. After careful study it is our conclusion that sound for TV should be independently equipped and operated. The TV project cannot establish standards and accept responsibility for quality of audio unless it controls the equipment and has sole responsibility for its operation. The costs involved are relatively small ($1,000) and the duplication seems justified in view of the real need for other jurisdictions to operate the existing systems on a daily basis.

The only other problems of importance relate to equipment and are discussed below.

Equipment

The Clinical Center system is relatively well equipped. We are making use of a black and white projector which is borrowed and should acquire one of our own. We lack facilities for incorporating slides and opaques into our presentations and are using a makeshift arrangement for presenting motion picture material. The central control room has been completely re-worked to increase its flexibility, but the control position has not been packaged to reduce its manpower requirements to a minimum because we lack funds for the special console housing needed.

We are using a great deal of old and some obsolescent equipment. A planned program of retirement of this equipment should be started on a modest basis. About $2,500 a year will be ample for this purpose. We should reduce our dependence upon Walter Reed for extra cameras by the purchase of an additional black and white camera chain as soon as staff to operate it is available. Supplies are now being drawn from a modest stock pile which will be depleted by late in the fiscal year. Most serious deficiencies will be in camera tubes which are expensive items. It should be noted that we have never had to purchase an orthicon camera tube during this project.

For research, our needs will be limited to funds for the purchase of relatively few items as any major items needed will be purchased by Institutes or are on hand. Supplies such as film and processing costs for film constitute the major expenses and these are relatively modest items.

Equipment for the new operating rooms will represent a major expenditure. One NTSC color camera is essential and at least two field sequential color cameras with associated receiving gear should be included in the first equipment complement.

Summary of equipment needs:

Clinical Center System:
(estimates are at probable purchase price rather than list)

GPL B&W large screen projector	$3,000.00
RCA Slide projector and multiplexer	4,000.00
Control Console housing	750.00
Retirement of obsolete amplifiers, etc.	2,500.00
RCA B&W camera, complete with acc.	17,000.00
Test gear	500.00
Total	$27,750.00

Research Activities

Optical equipment and light sources, etc.	$ 2,000.00

New Operating Suites

RCA or GE NTSC camera complete	$55,000.00
Large screen projection for above	on hand
2 GE field sequential cameras	50,000.00
1 large screen projector (eidophore)	17,000.00
Monitor receivers	2,000.00
Development of sterilizable camera head to meet CC requirements	5,000.00
Total	$129,000.00

Summary of needed supplies of types that should be stock-piled.

2 Orthicon tubes, RCA 5820	$ 2,400.00
Vacuum tubes (including Klystron and special)	1,000.00
Small components	1,000.00
Kine tubes - 10 @ $50.00	500.00
Total	$ 4,900.00

The CC system needs should be met as rapidly as funds become available. The research needs are also immediate. The equipping of the new Operating Room should be scheduled to coordinate with other factors connected with its opening. The funds for developing the sterilizable camera head should be made available at once.

Personnel

The most serious problem faced by the television program has been personnel. Qualified people with proper technical skills were not only hard to find, but also not easily interested in the salaries we could offer which are less than those in industry. This difficulty was compounded by the difficulty in qualifying candidates for employment under Civil Service. Registers of electronic technicians were available but the eligible people had no skills of the sort our activity required. This situation was resolved in part in July when we were able to obtain authority to certify (for temporary employment) candidates meeting our requirements. At present, the lack of funds for the project is the most serious difficulty, with salary level and a shortage of candidates somewhat less important.

It should be noted that our project has attractions for skilled technicians due to the variety of activities, comfortable working conditions and the informality of a small undertaking. The opportunity to display initiative and inventiveness is also appreciated. Our sister institutions, WRAMC and NNMC, have been successful in their staffing for reasons other than working conditions. They have enlisted men freely and restricted their civilian staff to a cadre for instruction of military personnel and for their technical supervision. These cadre people have been employed through Wage Board procedures which permit much higher pay levels than the CS classification.

In August we secured the services of an excellent man who, while lacking formal professional training in engineering, has a wide experience in television and television equipment design. We feel that he has the qualities needed for the leadership of our technical group. He is the only person supported by the television project. He has been assisted by a technician loaned by the Technical Development Section of the Heart Institute through an informal arrangement that may be terminated at any time. This man supplies a variety of skills in biology, optics, physics as well as a very good knowledge of television acquired through working with us in research since 1957. We are exploring the possibility of transferring this man to the project. This has the approval of the Heart Institute, but classification difficulties have not yet been overcome.

The Clinical Center System has been designed to conserve man power. Four men can operate most programs in contrast to the usual requirement of about twelve. Four full-time people are required, however, if the equipment is to be maintained in top condition, and if its operation is to be good.

We have met, in part, the deficiencies in our man power by using the services of technicians from local TV stations. Each program has had to be staffed by assembling several men who were off duty. This is a very unsatisfactory and inefficient. Each new man had to learn the building, the system, and our method of operating. Working in our laboratories and on our nursing units involves relationships and patterns of behaviour that must be carefully observed. Our own staff has had to do all the preparations for each undertaking and this has involved overtime work as much of this preparation is temporary cabling which cannot be done far in advance because it is unsightly and hazardous.

We attempted to improve this situation by using personnel from the Instrument Shop. This proved unsatisfactory but was a slight improvement. Their technicians have only an elementary knowledge in this field. They are not always available because they have other responsibilities which they must meet. The hourly cost is fifty per cent greater than the cost of local TV station personnel.

Our greatest concern over this situation is related to maintaining good standards of performance. Under present conditions undertakings which should be straightforward and simple and well within our equipment resources prove to be difficult and are not accomplished

without technical deficiencies. While major failures, other than in the sound system, have not yet occurred, the fear of an embarrassing failure is real and warranted.

We feel the project should be put on a sound basis as soon as possible. This seems even more urgently needed in view of the television equipment being introduced in patient care areas (Cardiac Catheter Lab and Radiology) and in the new Surgical Wing. Technical support for these areas will be needed.

With these facts in mind, and with acute awareness of the administrative problems involved, we have reviewed the financial needs of the project for calendar 1960 very carefully. If one makes the unrealistic assumption that technicians could be employed at once the following figures would apply:

Personnel

Four technicians for Clinical Center System	$28,000.00
One technician for research and development of Surgical Camera head	6,000.00

Other Objects

Film and Processing (CC System kinescopes)		$ 4,000.00
Prints of kinescopes		1,300.00
Components		2,000.00
Research (film, etc.)		900.00
Stockpiled supplies*		- -
	Total	$43,200.00

* These figures exclude all capital expense including retirement of obsolete equipment, and also omit items for major expendable supplies that can be stockpiled. These items are discussed under equipment.

The funds available in calendar 1960 are:

1960 budget balance (approximately)		$ 8,000.00
1961 budget request (6 mos. pro rated)		15,000.00
	Total	$23,000.00

To this we can add the funds Dr. Baruch has recommended be set aside for developing a special surgical camera head, if this recommendation is approved. The maximum available funds would be not more than $28,000.

In the effort to find a workable method of meeting our needs, a more realistic approach was then applied. It was presumed that authority to add one technical person in each quarter of 1960 had been granted and that a candidate was available. The other objects money was considered fixed. The result was the following:

Fiscal 1960: (last six months)

	Expense	Funds Now Available
Personnel	$ 8,750.00	
Other	4,000.00	
Total	$12,750.00	$ 8,000.00

Fiscal 1961: (first six months)

	Expense	Funds in Budget Request
Personnel	$13,000.00	$15,000.00
Other	4,000.00	
Total	$15,750.00	

Fiscal 1961: (last six months)

Personnel	$17,000.00	$15,000.00
Other	4,000.00	
Total	$21,000.00	

It can be seen that the sums required to fully staff the project would not require large increases in calendar 1960. The fiscal 1960 increase would be $4,750; the fiscal 1961 increase would be only $750 during the first six months and an additional $6,000 in the second six months. In 1962 the full recommended budget would be in effect at the level of $42,300 which is an increase of $12,300 over the 1961 request.

In view of the fact that it is unlikely that technicians will be available for employment on the first day of each quarter, the total new money needed for calendar 1960 may be somewhat less than the estimate of $5,500. This sum is the same order of magnitude as funds Dr. Baruch has recommended be given to the TV project to develop the new surgical camera head. Authorizing the employment of technicians on the suggested step by step basis would not produce a serious financial problem in 1960.

If the Scientific Directors would make available the costs contemplated for research during fiscal 1961 ($6,900) in July of 1960, the budget situation would be quite comfortable for that year.

The 1962 budget request would have to be increased by $12,300. It seems reasonable to expect that this can be successfully defended in view of the increased responsibility the New Surgical Wing will introduce.

Recommendation

It is recommended that the technical staff of the TV Unit be brought to it's minimal satisfactory level by granting authority to employ one additional technician in each quarter of calendar 1960. The financial implications have been discussed and sources of funds suggested.

Attachment II
Serial No. CC-0

PROGRAMS PRODUCED BY WNIH-TV
The Clinical Center System

January 22, 1958: Fifth Annual NIH Lecture

"Relation of Physical Science to Life Science: Communication Theory"

Dr. W. O. Baker
Vice President in Charge of Research
Bell Telephone Laboratories

In anticipation of possible overflow audience, the lecture was televised from Clinical Center main auditorium on the first floor to the 14th floor gymnasium where it was projected on a large screen. This first attempt to provide this kind of coverage and service was executed in a very successful manner.

Audience = 285

September 11, 1958: Clinical Center Quarterly Clinical Staff Meeting

"Application of Instrumentation"

Dr. George Z. Williams
Chief, Clinical Pathology Department - C.C.
and Staff

Demonstration via closed circuit TV from 4th floor Clinical Center to Clinical Center auditorium on first floor.

Audience = 143

September 23, 1958: NIH Orientation

"Metabolic Studies - (Use of Respiratory Chamber)"

Dr. G. Donald Whedon
Assistant Director - NIAMD
and Chief of Metabolic Diseases Branch
and Staff

Audience = 112

November 18, 1958: NIH Orientation

"Use of Germ-Free Animals in the Study of
Oral Disease"

Dr. Robert M. Stephan
Acting Chief, Clinical Investigations Branch-NIDR

Dr. Robert J. Fitzgerald
Assistant Chief, Laboratory of Microbiology-NIDR

Mr. John L. S. Hickey
Chief, Germ-Free Service Section
Sanitary Engineering Branch-DRS

Audience = 271

December 3, 1958: PHS Management-Orientation Conference

"Research at the Bench and in the Clinic"

Dr. Abraham Goldin, Head
Biochemical Pharmacology Section
Laboratory of Chemical Pharmacology-NCI

Dr. John J. Burns
Deputy Chief, Laboratory of
Chemical Pharmacology-NHI

Dr. James Stengle
Chemotherapy Service
General Medicine Branch-NCI

First use of television cameras on nursing unit.
Assisted by WRAMC-TV.

Audience = 55

February 10, 1959: NIH Orientation

"Research in the Service Laboratory"

Dr. Paul Schmidt and Staff
Section on Blood Bank -
Laboratory of Blood and Blood Products-DBS

Audience = 187

February 17, 1959: MEDICO Meeting

Clinical Center auditorium. Televised to 14th floor assembly hall.

Second televising of a meeting to care for overflow audiences.

Audience = 215

February 26, 1959: Institute for Advancement of Medical Communication
Joint Board of Science Education - Washington Academy of Science

"Germ-Free Animal Research and Technology"

Dr. Richard Horton
Laboratory of Germ-Free Animal Research-NIAID

Mr. John L. S. Hickey
Germ-Free Service Section
Sanitary Engineering Branch-DRS

Simultaneous pickup and projection to demonstrate the value of TV in giving a large audience a close-up of small objects inside a germ-free tank.

Demonstration of technique to IAMC and content presentation to high school science students.

Demonstration of use of various types of TV gear in medical education. WRAMC, NNMC, and Dr. George Z. Williams of NIH collaborated.

Audience - 70 IAMC
250 Science Students

April 21, 1959: NIH Orientation

"A Rehabilitation Program in a Research Setting"

Dr. David Fried and Staff
Rehabilitation Department - Clinical Center

Dr. Roger Black
Arthritis and Rheumatism Branch - NIAMD

Audience = 1[illegible]

May 4 and 5, 1959: Seventh Conference on Microcirculatory Physiology and Pathology

Preview of Exhibits (displayed on 14th floor of Clinical Center) by Television (main auditorium, first floor of Clinical Center).

First use of NIH color equipment and color projection. Program tested a new method of conducting scientific meeting. Exhibits were presented to the entire assembly via TV between scientific papers. A special time was set aside for visits to exhibits when interested people could discuss the subject matter in detail with the exhibitor. This eliminated the need to attend exhibits other than at the appointed time.

Audience = 150

June 4, 1959: Second Pan American Congress on Rheumatic Diseases

Dr. Joseph J. Bunim
Clinical Director - NIAMD

Dr. Kurt Bloch
Dr. George Ehrlich
Dr. William M. O'Brien
Arthritis and Rheumatism Branch - NIAMD

Application of technique used on germ-free program to the problem of presenting pathology in patients to a large audience.

Audience = 350

June 15 and 16, 1959: NNMC Medical-Dental Television Workshop

"Electronic Image Processing" exhibit displayed.

"Patient Observation and Clinical Research" - Dr. Murray C. Brown - live demonstration of color TV ophthalmoscope.

Demonstration of low-light level TV.

Audience = 200

June 18, 1959: NIH Orientation

"Research at Work - 'How Drugs Enter the Brain and It's Fluids: Clinical Problems, Pathology and Pharmacology'."

Dr. David P. Rall
Head, Clinical Pharmacology and Experimental
Therapeutics Service, General Medicine Branch, NCI

Dr. Louis B. Thomas
Head, Surgical Pathology and Post Mortem Service
General Medicine Branch, NCI

Dr. Edward Moore
Chemotherapy Service, General Medicine Branch, NCI

Mr. James Gaskins
Clinical Pharmacology and Experimental
Therapeutics Service, General Medicine Branch, NCI

Audience = 300

July 31, 1959: A Special Program for High School Science Teachers Attending Summer Institutes Conducted by Area Universities

A. Basic Research

Dr. Abraham Goldin
Head, Biochemical Pharmacology Section
Laboratory of Chemical Pharmacology, NCI

B. Clinical Research

Dr. Emil Frei
Head, Chemotherapy Service
General Medicine Branch, NCI

Closed circuit TV system utilized to show an example of integrated basic and clinical research in the treatment of cancer.

Composition of audience:

A.U. High School Science Teachers
Nat. Educ. Assoc. Spns. Wash. Seminar
    (Elem. and Second. Teachers)
Morgan State Teachers College - Baltimore, Md
    (Summer Science Tr.Prog.Second.School Student
U. of Maryland
    NSF - High School Science Teachers -
        Bio., Chem., Physics

July 31, 1959: Composition of audience - continued:

Howard U.
(High School Science Teachers -
Rad., Bio., Chem., Physics)

Audience = 370

September 11, 1959: A Special Program for Medical Students

"An Example of Integrated Basic and Clinical Research"(Patient with Xanthoma demonstrated).

Dr. Robert S. Gordon
Laboratory of Cellular Physiology and
Metabolism-NHI

Dr. Simeon Margolis
Research Associate
Laboratory of Cellular Physiology and
Metabolism-NHI

Dr. John Potts
Clinical Associate
Laboratory of Kidney and Electrolyte
Metabolism-NHI

Program was introduced by Dr. Luther Terry, Assistant Director, NHI

Composition of audience:

Freshman class of G. W. Univ., plus students (all four class levels) from medical schools in the D.C., Md., Va., Penna., W.Va. area.

Audience = 150

September 25, 1959: Operation "K" - Visit of the Chairman of the Council of Ministers of the USSR

1. Gout

Dr. Joseph J. Bunim
Clinical Director, NIAMD

September 25, 1959: Operation "X" - continued

2. Metabolic Diseases

Dr. DeWitt Stetten, Jr.
Associate Director in Charge
of Research, NIAMD

3. Leukemia Ward

Dr. Emil Frei
Head, Chemotherapy Service
General Medicine Branch, NCI

4. Choriocarcinoma

Dr. Roy Hertz and Staff
Endocrinology Branch, NCI

5. Germ-Free Laboratory (Sub-basement of C.C.)

Dr. Walter L. Newton
Chief, Laboratory of Germ-Free Animal
Studies, NIAID

Mr. John L. S. Hickey
Chief, Germ-Free Service Section, DRS

This presentation tested the capacity of the Clinical Center system to handle elaborate TV productions and demonstrated the value of TV for presenting complete event to a large audience from a variety of locations to which they could not be admitted.

17 cameras, 2 projectors, a microwave, and 2 Kine recorders were involved.

Audience = 300

September 29, 1959: Ninth Symposium on Recent Developments in Research Methods and Instrumentation.

"Biophysical Methods Based on Electromagnetic and Electrokinetic Effects"

Dr. Alexander Kolin
University of California

September 29, 1959: Ninth Symposium on Recent Developments in Research Methods and Instrumentation = continued

TV camera and TV projection were used for this section to give a large group a "close=up" of intricate devices.

Audience = 226

October 6, 1959: PHS Management=Orientation Conference
NIH Orientation

"Galactosemia"

Dr. Yale J. Topper
Laboratory of Biochemistry and
Metabolism, NIAMD

Dr. Stanton Segal
Clinical Endocrinology Branch, NIAMD

Audience (PHS) = 55
(NIH) =233

October 15=16, 1959: Council on Medical Television

First meeting of the Council on Medical Television which is a sub=unit of the Institute for the Advancement of Medical Communication (IAMC). The Council is composed of representatives drawn from the following areas:=

Broadcast Industries
Medical Schools
Medical Professional Organizations =
  e.g., College of Physicians and Surgeons
Foundations = e.g., Ford Foundation
Drug Industries
National Voluntary Health Organizations =
  e.g., American Heart Association, American
    Cancer Society.

On morning of October 15 the Hon. Arthur S. Flemming, Secretary of HEW, delivered keynote address via closed circuit telecast from his office, DHEW to Clinical Center auditorium, Bethesda.

This was the first time that the NIH group microwaved a program from a remote location.

Audience = 80

October 16, 1959: MEND Symposium - Preventive Medicine and Health Mobilization

Afternoon: Orientation to NIH for Professors of Preventive Medicine - Dr. Murray C. Brown, Chief of Clinical and Professional Education, presiding.

3:30: "Television Tour of Research in Progress" - Dr. Dorland Davis, Associate Director in Charge of Research, NIAID, presiding:-

(TV) The Clinical Use of Frozen Red Cells

Dr. Paul Schmidt
Chief, Blood Bank Section, DBS

(Color Kinescope and $3\frac{1}{4}$" x 4" slides) - Effects of Radio Waves on Rhesus Monkeys

Dr. Maitland Baldwin
Chief, Surgical Neurology Branch, NINDB

(B & W Kinescope) Use of Germ-Free Animals in Research. Presented (live) by -

Dr. Walter L. Newton
Chief, Laboratory of Germ-Free
Animal Research, NIAID

(TV and $3\frac{1}{4}$" x 4" slides) - Fungal Endocarditis

Dr. John P. Utz
Chief, Infectious Diseases Service,
NIAID

Dr. Howard M. Kravetz
Clinical Associate
Infectious Diseases Service, NIAID

Note: Members of the Council on Medical Television, whose meeting concluded at 12:00 noon, were invited in to the afternoon session of the MEND meeting on the basis that the application of TV would be meaningful and interesting to them.

Audience (Combined) = 150

October 17, 1959: High School Science Teachers

(NIAMD and NCI - Note: Primary relationships with this office, NIAMD)

"Enzymes and What They Do and Metabolic Diseases"

Dr. Bert La Du
Arthritis and Rheumatism Branch, NIAMD

Dr. Robert Fouty
Resident
Clinical Pathology Department, C.C.

"Capillary Circulation"

Dr. Louise H. Marshall
Laboratory of Physical Biology, NIAMD

Note: "K" operation Kine - Dr. Bunim - Gout Patients - Some background of "K" Kine preceded.

Evaluation made by NIAMD very complimentary re TV.

Audience = 200
plus approx. 25 NIH staff

November 24, 1959: Clinical Center Pharmacy (Milton Skolaut)
Presentation for Maryland Association of Hospital Pharmacists.

Audience = 125

December 15, 1959: PHS Management-Orientation Conference
NIH Orientation

"Hematology"

Dr. N. Raphael Shulman (Head)
Dr. Thomas C. Bithell
Dr. Alfred Leitner - Clinical Associate
Dr. Richard Astor - Clinical Associate

Hematology Group
Clinical Investigations, NIAMD

Laboratory and patient ward (8W) visited.

December 15, 1959: PHS Management-Orientation Conference
NIH Orientation - Continued

"Hematology" - continued

Dr. Russell Little
Assistant Hematologist
Hematology Service
Clinical Pathology Department, C.C.

(By B & W Kinescope)

Dr. Paul Schmidt
Chief, Section on Blood Bank
Laboratory of Blood and Blood Products, DBS

(By B & W Kinescope)

Audience -	55 (PHS)
	146 (NIH)
	180 (High School Students) 1/

1/ Stone Ridge Country Day School
Bethesda, Maryland

Academy of the Holy Names
Silver Spring, Maryland

Parkville Senior High School
Baltimore, Maryland

Calendar Year 1959

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER
ADMINISTRATIVE BRANCH

PATIENT ACTIVITIES SECTION

CC-05
Serial No.

The Patient Activities Section of the Administrative Branch, Clinical Center, comprises a number of organized activities executing a broad lateral series of responsibilities directed to the diversional interests of the patients. It is responsible for the administrative management of the Normal Volunteer Program, and represents the Clinical Center Director's office in the administration and guidance of the policies established by the medical board of the National Institutes of Health.

During calendar year 1959 this organization continued to carry out its primary functions:

1. To provide a volunteer service program.

   A. The Grey Lady Service. The American Red Cross Grey Ladies supply personnel to carry out the responsibilities beyond those discharged by the employees of NIH.

   B. The Pool of Bethesda is an executive organization composed of many religious and fraternal groups in the Bethesda and Washington areas. They donate such items as bingo prizes, party decorations and small Christmas gifts for patients.

2. To provide a Patient Library Service.

3. To provide recreation in both social and adapted sports.

4. To provide a Normal Volunteer patient program.

5. To provide a childrens program.

Recreation

The diversional program was placed within the responsibilities of the recreation leader and includes sports, both active and passive in nature, with spectator participation; and a broad program of social activities. In the selection of activities, it is essential that the patient have medical approval to insure compatibility with the individual tolerance levels and the patients role in his research study.

In the area of recreation several problems have arisen, primarily because of the lack of experienced personnel. This has imposed a heavy burden of responsibility on our clerical staff. It has not only limited its progress, but has forced us to discontinue several of the basic activities. The severity of this problem has become more acute with the resignations of our Recreation Supervisor, Mr. Lewis Wills, and Miss Mary Ann Porterfield, Recreation Assistant. The addition of Mr. George Lee, Recreation Leader, to our staff in December has not alleviated our supervision problem, nor has it provided staff for the childrens program.

The patients and normal volunteers are provided supervision to cover their daily activities. Each morning between 10:00 and 12:00 a.m. the gymnasium is reserved for the Mental Health Institute. We do not assume direct responsibility for the supervision in their recreation program. However, they frequently request our assistance, and in most cases we extend this courtesy.

The gymnasium is open daily between 1 and 2 p.m. for patients needing specific supervision in the NINDB. Between 2 and 3 p.m. all medically cleared patients from other institutes participate in a general interest program. The normal volunteers participate in activities between 3 and 5 p.m., and this time is divided into two phases. Between 3 and 4 p.m. the volunteers participate in general interest activities, and between 4 and 5 p.m. the male volunteers devote their time to basketball. Daily, between 5 and 7 p.m. the Recreation and Welfare Association has the gym reserved for the employees.

Tentative plans include sport activities outside of the Clinical Center, when weather permits. Since the golf course at Glenbrook will be inactivated, due to construction, it is important that consideration be given to an area for developing a playground.

The social phase of the recreation program involves parties, spectators activities (live shows, games, motion pictures, dramatics, hobbies and arts and crafts etc.). In addition, we continue to schedule a craft program Monday night for normal volunteers.

However, the title of this program is misleading because the work involves the use of the hands and mind. On occasions the volunteers participate in craft activity. The program also includes lectures on subjects of general interest. The fundamental reason for this type of program is to broaden the intellectual scope of the normal volunteer.

We have had a number of very fine shows during the past year. Depending on the time of the show and the type, the audience varies in number and reaction. More good shows would be desirable, but where tohold them, and how to obtain them presents quite a problem. At least one staff member is necessary to greet the performers and run the backstage light-board.

Parties

There have been two parties, one was for Valentine Day, with 107 attending. It was not very successful, mainly because of taped music. On Halloween, 178 attended a party that was a great success. We had a band and the patients enjoyed it emmensily.

Movies

The children perfer adventure, comedy, or cowboy films. The attendence is about 30% adult.

Attendence has almost doubled since conversion to cinemascope because of a wider and better selection of movies. This is particularly noticable at the evening showing.

Children's Program

The Children's Program was started in March 1958 with 3 boys on 9-West. In April we began taking on children from other units. Activities were limited by lack of supplies. Since then, we have covered all units with the exception of 2-East, and now have quite a good supply of material with which to conduct the program. The Normal Volunteer patients are now assigned to assist in the program, and this has worked out quite well.

Activities

Swimming Pool
Walks on Reservation
Toys to ride
Play house set up in 14th floor solarium
Table games
Gym activities
Movies

Normal Volunteer Program

Administrative management of the Normal Volunteer Program was placed in this section to maintain financial, personnel and activity records; to insure observance of the basic rules governing utilization in research and educational activities, and to maintain liaison between the five agencies under contract to supply the volunteer subjects.

Since its inception in 1954, when 17 volunteers contributed 1278 days of service, we have shown a steady increase. In 1959, 100 volunteers contributed 10,628 days of service. To date, 404 volunteers discharged from the Clinical Center have contributed 41,295 research days.

Geographically, the volunteers arrive from over half of the United States and occasionally from foreign countries. However, the greater percentage is from Pennsylvania, Ohio, Illinois, and Indiana. The average age for 1959 was 21.3 years. However, this includes one ofthe older volunteers who participated in a specific project demanding a subject in this age group. When this volunteer was excluded the average age decreased to 20.8 years.

Appreciable strides have been made towards our goal of having supply exceed demand. Discharge of volunteers in 1959 showed an increase of 15 per cent over 1958. With the addition of three new contracts (Bennington College, Maryland University and American University), and our continuing contracts with the Brethren and Mennonite churches; we have managed to prevent an acute shortage. Moreover, we anticipate negotiating with several new sources in the near future, and contemplate an adequate supply of normal volunteer patients. This should relieve us of the pressures we experienced when demand exceeded supply.

Patients' Library

Accomplishments during theyear: During several months of the year 1959, the Patients' Library conducted the most extensive program of its 5-year existance. Besides weekly bookcart visits to patients on each nursing unit (except NIMH), and delivery of special requests and new magazines every Friday, the staff carried on these activities:

1. Weekly reading groups on 2W, 3E, 3W, 13W.
2. Weekly discussion group, 3E, 3W.
3. Weekly story hour for all children in the Clinical Center.
4. Reading and story telling to individual children or small groups, as part of bookcart visit to ward.
5. Improved individual service to eye patients and blind patients, using Talking Book machines and records.

6. Individual service to patients using other reading aids, such as the Automatic Page-Turner.
7. Occasional scheduled library hours for groups of mental health patients.
8. Attendance at staff and staff-patient meetings, especially in NIMH.
9. Employment of mental health patients as part of their therapeutic program. One patient has worked an average of two hours a day throughout the year; another worked an hour or two a day for 6½ months before being discharged; two others worked for shorter periods.
10. National Library Week was celebrated from April 13-19. Besides an exhibit on the 14th floor, posters were displayed on each nursing unit and elsewhere in the Clinical Center. A committee of NIH staff members helped plan the Reading Night program, and 13 of them conducted reading and discussion groups with patients in each solarium. Through the cooperation of the Dietetic Department, special National Library Week place mats were used on patients' trays for one meal, and library week programs and book marks were also distributed in this way. An open house was held in the Patients' Library one day to demonstrate reading aids and display new books. Patients and staff members were invited.

    Highlighting patient library service in a dramatic way for a week each year results in greater use of the library, and seems to have a beneficial, socializing effect on some wards. Weeks after the celebration, patients will tell us how much they enjoyed the reading night, and ask if we plan to have any more such meetings. It also makes the staff more aware of the services available, and we notice even greater cooperation than usual for sometime after library week.
11. To publicize the library, a weekly column is printed in the Patient Activities Schedule. Weekly talks are given to new nursing employees; orientation talks are sometimes given to new social workers, occupational therapists and others. The librarian explains library service to new Gray Lady classes and demonstrates equipment available to patients. As needed, training classes are held for Gray Ladies working in the library; for instance a one day refresher course was held in November and a training class for new Gray Ladies assigned to the Library is being planned.

12. Even though gifts to the library do not fit into this paragraph, it should be noted that the Patients' Library regularly receives many gifts--hard covered books, paper-bound books, and magazines. During 1959, two patients donated cash ($25 and $15), three foreign patients made gifts of books, and several people gave subscriptions to magazines.

Major problems continue to be lack of space and staff and staff needed to develop the library program more effectively. Even with our small staff, our lack of adequate space--work-room, office, storage, reading room--is a serious handicap in carrying out our duties. We find that lack of regular communication with departments providing services for patients continues to hamper our efforts to coordinate our services with those of others; to avoid duplication and over-lapping of work, and to operate as part of a team.

During 1959, the assistant librarian resigned, and there was a great deal of sickness and absenteeism for other reasons on the part of the Gray Ladies. This necessitated curtailment of our services during the summer. A new librarian reported for duty in September, and two new Gray Ladies have been assigned to the library. With this help we are gradually adding services that had to be dropped.

Proposed future objectives

1. Establishment of a monthly "Reading Night" or literary program for interested patients. This has been planned with staff members and outside guests who have helped with the 1958 and 1959 celebrations of National Library Week, but because of lack of library staff has not been started yet.
2. Development of a Talking Book program for patients not able to read regular books because of a disability, but not eligible to use the free Talking Books available to the blind. We now have a collection of twenty recordings and a new, light-weight, reasonably priced machine which we are trying out before ordering more. Any patient, of course, will be allowed the machine and records, but preference will be given to those with disabilities interfering with ordinary reading.
3. Improved service to partially-sighted patients. We already lend magnifying glasses where they are needed, and are starting a collection of books in large print (18 and 24 points.)
4. Resumption of reading and discussion groups are needed or desired. (At this writing, only one group, on 2W, meets weekly.)

Calendar Year 1959

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER
OFFICE OF THE DIRECTOR

CC-09
Serial No.

CHAPLAINCY DEPARTMENT

PROTESTANT CHAPLAIN

This year a study was concluded, entitled "Program and Personnel Needed to Meet the Religious Needs of the Protestant Patients in the Clinical Center." It states, in summary:

The following findings indicate a serious deficiency in the religious care that is provided the Protestant Patients in the Clinical Center:

1) Three-fourths of the Protestant patients who desire a chaplain's services do not receive any.

2) The 40 man-hours per week now provided for the spiritual care of Protestant patients is less than 8 minutes per patient per week. This is not enough time even to provide an emergency ministry to patients who are seriously ill, in major surgery and specially referred, which would require 11 hours per day.

3) A Protestant Program that would meet national standards, as well as the special needs of the Clinical Center, would require about 300 man-hours per week, or 1 hour per patient.

These findings are confirmed by a survey of chaplains in leading general hospitals. They are in keeping with the expressed goal of the N.I.H. Clinical Center, to bring the best that modern medicine and its ancillary services have to offer to the care and rehabilitation of the patients. It is especially true for patients caught in "incurable" diseases that their need for spiritual care is equal to their need for physical care.

To provide 1-man-hour per patient per week to meet the spiritual needs of the patients in the Clinical Center is modest, in comparison to the thousands of man-hours per week devoted to the study and care of their physical needs.

To provide adequate religious care requires six additional chaplains.

Provision for one additional chaplain has been made in the 1961 budget. This will make it possible to provide a minimum, emergency ministry to Protestant patients, but no more.

SUMMARY OF SERVICE - Protestant

January thru November - 1959

Ministry to Patients

Initial interviews	904
Counseling	76
Rounds	1,110
Criticals	1,057
Baptisms	3
Funerals	2
Weddings	1
Pre-Oper.	529

Worship

Chapel Service	50
Attendance	3,651
Average	73

Holy Communion	13
Chapel	542
Bedside	176

Patient Referrals

Doctors	52
Nurses	636
Social Workers	119
Clergy	38
Family	259
Patient	54
Others	40

Staff Relations

Counseling	21
Conferences	847
Staff Mtgs.	14
Lectures	37

Community

Addresses	8
Services	16
Meetings	21
Professional Conferences	26

Administration

Correspondence	714
Planning	344 hours
Conferences with chaplains	49
Study and Research	250 hours

CATHOLIC CHAPLAIN

I. The Catholic chaplain has been on full-time duty at the Clinical Center since June, 1958. Services provided by the Catholic chaplain are summarized below in a general manner, followed by a detailed summation of daily activities for the Calendar Year 1959 through the 13th of December.

With an average Catholic patient census of eighty (80), it requires of the Catholic chaplain an average of sixty (60) hours a week to adequately fulfill his duties. Besides these routine activities there are matters of special interest which it may be well to mention in this report.

The first matter of special interest concerns the establishment of a Catholic Chapel. To fill the needs of Catholic patients in the most suitable manner, there should be a place in the hospital where the Blessed Sacrament (Holy Eucharist) may be reserved. This Sacrament, according to Catholic Worship, must be reserved in a Chapel used exclusively for Catholic Worship.

The need for such a Chapel may be more easily understood when it is realized that the Blessed Sacrament is the very center and essence of Catholic worship. Under normal circumstances, it is administrated to Catholics usually only during their attendance at Mass. But it is also administered to an individual Catholic in time of critical illness as a part of the Last Rites of the Catholic Church. If there is no place in the hospital to reserve this Sacrament for such occasions, it is necessary for the chaplain to go out to one of the Catholic Churches in the community to obtain it. In some instances there isn't time for him to do this. A further need is filled by such a Chapel, since it is a custom of Catholics to make visits of devotion to the Blessed Sacrament in its place of reservation.

At the present time plans for such a Chapel are nearing completion. A small room has been provided temporarily until more suitable space becomes available. Bids for the furnishings have been received and approved. Sometime shortly after the first of the year this Chapel will be ready for use.

A second point of interest to be noted is the continually increasing requests by personnel for appointments with the chaplain. The purpose of these requests is almost always for personal reasons. It has been the practice of the Catholic chaplain to make such appointments, determine the reason why the person sought the appointment, and then after some brief counsel and advice, to endeavor to refer the person to the priests in their own parish. In many cases, this solution is fine, but in many others the person have a problem that stems partially from the nature of their work at N.I.H. As a result it is not easily understood by outside clergymen and must be handled by the chaplain. All of these requests by personnel are time consuming.

CATHOLIC CHAPLAIN

II. GENERAL SUMMARY OF SERVICES PROVIDED BY THE CATHOLIC CHAPLAIN

Divine Worship Service (Mass) for patients and personnel daily
(two services on all Sundays and Holydays)

Special religious services daily during months of May and October.
Special religious services as scheduled during seasons of Advent and Lent.
Sacraments administered to patients daily.
Participation in Orientation program of all new Nursing and Social Service personnel.
Participation in Gray Lady Training Program.
All patients visited on a regular schedule for counselling.
All patients visited each Saturday for confession.
Seriously-ill patients visited every day.
Weekly consultations with families and patients.
Consultations with Staff regarding welfare and rehabilitation of Patients.
Consultations with personnel on request.
On call for emergency service twenty-four hours a day.

III. DETAILED REPORT OF DAILY ACTIVITIES

For January 1, 1959 thru December 13, 1959

I. CHAPEL SERVICES

Masses	369
Attendance	9,436
Sermons	44
Holy Communion	10,908
Chapel	4,157
Bedside	6,751
*Devotions	53
Attendance	2,196

II. MINISTRY TO PATIENTS

Wards Visited	4,500
Initial Interviews	490
Counseling	1,101
Seriously ill	1,658 visits
Pre-Ops.	182
Confessions	1,270
Chapel	36
Bedside	1,234
Baptisms	3
Confirmations	1
Last Rites	72
Funerals	1
Follow-ups	106

III. PATIENT REFERRALS

Doctors	368
Nurses	647
Social Workers	67
Clergy	66
Family	667
Patient	75
Others	129

IV. STAFF RELATIONS

Counseling	338
Conferences	2
Staff Meetings	9
Lectures	39

V. ADMINISTRATION

Correspondence	198
Planning	30 hours
Conference with Chaplains	6
Study & Research	104 hours

VI. COMMUNITY

Addresses	2
Meetings	2
Professional Conferences	0

* Devotions

February and March - Lenten Devotions
May and October - Rosary Devotions

JEWISH CHAPLAIN

Jewish chaplaincy service is on a contract basis providing for twelve visits per month. Rabbi Joseph M. Brandriss has been serving as Jewish Chaplain since November 24, 1956. Approximately 8% of the patients admitted to the Clinical Center are Jewish.

SUMMARY OF SERVICE

Sabbath services on Fridays and special service for the various major and minor Holidays of the Jewish calendar, including some socials after the celebrations.

Visits to patients.

Counseling of patients and families.

Religious education when requested and/or needed.

On call for emergency service twenty-four hours a day.

Calendar Year 1959

PUBLIC HEALTH SERVICE, NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER
ADMINISTRATIVE BRANCH

EMPLOYEE HEALTH SERVICE BRANCH

CC-12
Serial Number

Major Goals

The Employee Health Service Branch of the Clinical Center provides occupational medical services to employees under existing laws and regulations, and acts as the focal point for all matters relating to the health of employees. The Employee Health Service has a dual responsibility; first, to management, to assist in the prevention and control of occupational injuries and diseases, to reduce sickness absenteeism, and to improve working efficiency and productivity; second, the Employee Health Service has a responsibility to employees to provide prompt and effective treatment for those occupational injuries and diseases which do occur in spite of preventive efforts and in general to assist them in maintaining their health at the highest possible levels consistent with accepted preventive health practices, by offering such services as immunizations, health advice and guidance and periodic physical examinations.

The Employee Health Service recognizes its responsibility to contribute to nation-wide efforts to improve the practice of Occupational Medicine; and confidently hopes to merit continued management support which has enabled the NIH program to stand out as a model for other employee health programs both in government and private industry. Because of the relatively stable employee population working under controlled conditions, the Employee Health Service has an excellent opportunity to develop the research potential of Occupational Medicine, and will continue to collaborate with NIH scientists in pursuit of appropriate projects.

Major Progress

Efforts to provide day-to-day services to employees on a more personalized basis have been made over the past year. The staff has been encouraged to include more individual health counselling in its routine day-to-day contacts with employees.

Major Progress (Continued)

Impersonal mass screening procedures have been de-emphasized to some extent, with the exception of an expanded immunization program.

Continued awareness of the importance of controlling occupational disease hazards in the environment has prompted the Employee Health Service to increase the coverage and extent of its immunization program. Small-pox immunization was offered to all employees in the past year, and particular groups of employees with special health hazards are being offered other appropriate immunizations.

The Environmental Health Advisory Group, comprising Plant Safety, Sanitary Engineering, and Employee Health activities has continued to function collaboratively in an effort to control environmental health hazards. The informal working relations developed by these activities in the control of many health hazards has proved satisfactory and will be continued in the coming year.

In an effort to improve the working relationships between various levels of management and the members of our staff, consistent efforts have been made to assist supervisors in the evaluation of health problems in their employees which presumably could be influencing satisfactory work performance. Procedures for obtaining "Fitness for Duty" examinations have been simplified, partly because of the increased demand for this type of examination and the realization that an early examination of this type may be more beneficial both to the employee and to management than a long delayed one.

The research potential of Occupational Medicine, as specifically applied to the NIH working population, was demonstrated during the year with the acceptance for publication in the AMA Archives of Industrial Health of the paper "Reactions to Influenza Vaccine Inoculations". In addition, the Employee Health Service has assisted in many clinical research projects through early case finding and referral. As in the past, we stand ready to assist any NIH scientist in the proper utilization of the employee population in suitable research projects.

Major Progress (Continued)

Cooperation with private practitioners in the community has continued on the same high level which has existed during previous years. Constant surveillance is necessary to maintain these relationships which are at times in delicate balance.

Major Problems

In spite of rather extensive alterations to provide additional rooms within our existing space, we still are in need of additional space to provide the personalized type of service which we feel is so important. We continue to be hopeful that progress is being make in obtaining this additional space for the Employee Health Service in the area of B2A level now occupied by the Laboratory Aids Branch.

Selected Statistics

	FY 1959	1958	1957	1956
Total Visits..............	27,579	28,014	28,646	23,339
Visits for Occupational Injuries and Diseases...	3,138	2,600	2,896	2,960
Immunizations...........	15,604	13,154*	8,760	3,009
Pre-emp. Physical Examinations.............	1,704	1,828	1,637	1,915
Laboratory Examinations.	12,332	12,868	6,528	5,945
Referrals to Personal Physicians.............	1,736	1,631	1,454	1,353

*Recorded in error in 1958 as 8,234 total immunizations.

Calendar Year 1959

PUBLIC HEALTH SERVICE, NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER
ADMINISTRATIVE BRANCH

EMPLOYEE HEALTH SERVICE BRANCH

CC-12
Serial Number

Major Goals

The Employee Health Service Branch of the Clinical Center provides occupational medical services to employees under existing laws and regulations, and acts as the focal point for all matters relating to the health of employees. The Employee Health Service has a dual responsibility; first, to management, to assist in the prevention and control of occupational injuries and diseases, to reduce sickness absenteeism, and to improve working efficiency and productivity; second, the Employee Health Service has a responsibility to employees to provide prompt and effective treatment for those occupational injuries and diseases which do occur in spite of preventive efforts and in general to assist them in maintaining their health at the highest possible levels consistent with accepted preventive health practices, by offering such services as immunizations, health advice and guidance and periodic physical examinations.

The Employee Health Service recognizes its responsibility to contribute to nation-wide efforts to improve the practice of Occupational Medicine; and confidently hopes to merit continued management support which has enabled the NIH program to stand out as a model for other employee health programs both in government and private industry. Because of the relatively stable employee population working under controlled conditions, the Employee Health Service has an excellent opportunity to develop the research potential of Occupational Medicine, and will continue to collaborate with NIH scientists in pursuit of appropriate projects.

Major Progress

Efforts to provide day-to-day services to employees on a more personalized basis have been made over the past year. The staff has been encouraged to include more individual health counselling in its routine day-to-day contacts with employees.

Major Progress (Continued)

Impersonal mass screening procedures have been de-emphasized to some extent, with the exception of an expanded immunization program.

Continued awareness of the importance of controlling occupational disease hazards in the environment has prompted the Employee Health Service to increase the coverage and extent of its immunization program. Small-pox immunization was offered to all employees in the past year, and particular groups of employees with special health hazards are being offered other appropriate immunizations.

The Environmental Health Advisory Group, comprising Plant Safety, Sanitary Engineering, and Employee Health activities has continued to function collaboratively in an effort to control environmental health hazards. The informal working relations developed by these activities in the control of many health hazards has proved satisfactory and will be continued in the coming year.

In an effort to improve the working relationships between various levels of management and the members of our staff, consistent efforts have been made to assist supervisors in the evaluation of health problems in their employees which presumably could be influencing satisfactory work performance. Procedures for obtaining "Fitness for Duty" examinations have been simplified, partly because of the increased demand for this type of examination and the realization that an early examination of this type may be more beneficial both to the employee and to management than a long delayed one.

The research potential of Occupational Medicine, as specifically applied to the NIH working population, was demonstrated during the year with the acceptance for publication in the AMA Archives of Industrial Health of the paper "Reactions to Influenza Vaccine Inoculations". In addition, the Employee Health Service has assisted in many clinical research projects through early case-finding and referral. As in the past, we stand ready to assist any NIH scientist in the proper utilization of the employee population in suitable research projects.

Major Progress (Continued)

Cooperation with private practitioners in the community has continued on the same high level which has existed during previous years. Constant surveillance is necessary to maintain these relationships which are at times in delicate balance.

Major Problems

In spite of rather extensive alterations to provide additional rooms within our existing space, we still are in need of additional space to provide the personalized type of service which we feel is so important. We continue to be hopeful that progress is being make in obtaining this additional space for the Employee Health Service in the area of B2A level now occupied by the Laboratory Aids Branch.

Selected Statistics

	FY 1959	1958	1957	1956
Total Visits	27,579	28,014	28,646	23,339
Visits for Occupational Injuries and Diseases	3,138	2,600	2,806	2,960
Immunizations	15,604	13,154*	8,760	3,009
Pre-emp. Physical Examinations	1,704	1,828	1,637	1,915
Laboratory Examinations	12,332	12,868	6,528	5,945
Referrals to Personal Physicians	1,736	1,631	1,454	1,353

*Recorded in error in 1958 as 8,234 total immunizations.

Serial No. [illegible]
Calendar Year [illegible]

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

SOCIAL SERVICE DEPARTMENT

Trends:

During the past year the Social Service Department has been fortunate in having a full complement of staff much of the time. When vacancies did occur they were filled in shorter periods of time than in the past, which may indicate greater interest in obtaining employment in this department, since the personnel situation in clinical social work has not changed to any great degree. By having the full complement available, the department has provided adequate coverage for patients needing social service assistance and has met the special needs of certain projects which require 100% coverage. (See Table I)

Of special significance during this year is the growing number of patients from different cultural groups, such as Indians, Eskimos and natives of foreign countries, who not only present problems of language but whose different cultural backgrounds require a high degree of skill from the social workers serving them. For instance, a social worker in the Heart Institute has an important role with the children from Alaska who are sent to the Clinical Center by the Indian Health Service for heart surgery. During the past year five children have been admitted, the youngest being 2 years of age and the oldest 16. The worker is the liaison person with the Indian Health Service, and facilitates travel arrangements at the time of admission and discharge. The children are here without their families, and even written communication during their hospitalization is impossible for some. The social worker gets to know the youngster at the time of admission, and is the person who has a consistent continuing relationship with the child throughout his stay. Her purpose is to help the youngsters with the experiences they are having in the hospital, and to help with those problems appropriate for the caseworker to handle. Often, the primary work in the pre-operative period is to relieve the feelings of anxiety and fear which may be unspoken and acted out. Following surgery, the worker tries to prevent the repressing of post-operative fantasies, in order to minimize psychological residue which

might adversely affect the patient. Dealing with separation anxiety, particularly with the younger children, is a major ingredient of the case-work treatment.

Another patient with an unusual background is a three-year old Navajo Indian child, daughter of a tribal medicine man, who was referred by the U.S.P.H.S. Hospital, Tuba City, Arizona. Since she had her second birthday shortly after reaching the Clinical Center, the patient had little difficulty in adjusting as far as cultural patterns are concerned. However, the very different culture of the patient's parents has necessitated certain techniques and sensitivities in our correspondence with the patient's parents. For instance, in writing them a letter about the patient's progress, we mentioned that she was learning to talk, thinking they would be glad to hear of her general development. However, by return mail the family indicated concern that the patient is learning English, not Navajo. In this same case, while it is generally important to send pictures of children to parents who can visit the hospital or not at all, infrequently there is an additional special reason for sending pictures to this patient's family. The Navajos are a practical, simple people who believe what they see, and distrust intangible evidence such as a letter merely stating that Alice is all right. Therefore, pictures sent periodically give them concrete proof that the patient is being well-treated, and is thriving under the hospital's care. As we learn additional facts from the social worker on the reservation about what is important in the Navajo culture, we try to adapt our work with the parents accordingly.

Analyses of the statistics of the department again show that approximately 2/3 of staff time is devoted to direct services to patients and their families; and 1/3 in professionally related activities such as Institute staff meetings, ward rounds, teaching rounds and community activities of the NIH, PHS and the larger professional community of this area. (See Table IV)

As noted in the last annual report, there continues to be an increasing use of social workers in directly related activities of research projects, where their training and experience equip them to make observations in the social or emotional spheres which have direct value to the research goals.

Throughout the profession of clinical social work, staff members are becoming known as having an intensive and extensive experience with particular disease categories, and are increasingly being called upon to participate in professional seminars, institutes or similar meetings to provide others with the specialized knowledge they have acquired in their service at the Clinical Center. To make these

high school records and using the criteria of academic achievement in the upper half of the class; positive personality ratings of teachers; extracurricular activities; and the geographic location of the college which the student planned to attend. The caseworker made the original contacts with the students and set up the schedule for interviewing, but each member of the team interviewed some of the students. Parents of the students were interviewed in their homes.

Part of the student study has been done in the colleges where the students are now enrolled. Each member of the team visited the students he had previously interviewed. Plans are to visit the students in their homes on their Christmas leave, and again at the end of the school year, as well as to continue seeing families in the home.

In the Personality Development Study, one caseworker, assisted half-time by another, is responsible for screening approximately 30 families per month until July 1960, to obtain six who meet the study criteria of an experimental nursery school. The average stay of the children is four weeks, and in order to obtain a steady flow of families to maintain the project requirements and to set up a reserve, the two caseworkers are almost constantly out in the community. In addition to seeing the parents in the homes, contacts are had with the Board of Education, school principals, and churches, to search school records for potentially eligible families, and to discuss the aims of the project and the children's welfare.

One of these caseworkers is also responsible for obtaining a selected number of young married people for the Couple Study. This entails establishing good relationships with the Clerk of the Marriage License Bureau, so that records will be made available; and to visit the homes of prospective study couples at night when both members are present. In his role as caseworker for the completed Children's Service project, the same caseworker does the follow-up on the children, and is the liaison person for the Clinical Center. He visits the institutions in New Hampshire and New York where several of the children were placed; and the Maryland State Training School, to which one of the children was returned; and reports to the project administrator here on their progress and any new developments.

The caseworker for Family Relations in the Schizophrenia project visited for a week in the community of one family that lives 300 miles distant, in Radford, Virginia. She had many community contacts with the church, schools, townspeople, neighbors, etc., and recorded many of the interviews. These were reported on to the

project staff, and were very valuable in teaching more about family operations in their natural setting.

Two caseworkers, with one staff member from another discipline, visit several of the families under study; for dinner, for social evenings, etc., to observe patient and family interaction. This is atypical of the more traditional home visit where the social worker visits alone, when the patient usually is not present, and where the length of the visit is approximately one hour with a structured purpose, i.e., social and background data.

The program supervisor of this Section, together with one of the staff psychiatrists, made preadmission visits to the homes of prospective patients and families for screening and selection purposes.

Staff Development:

The Intramural Program:

During 1959, three major areas of interest were fostered and integrated within the intramural staff program:

1. The Casework Workshop on Psychosocial Diagnosis.
2. The Research Orientation Lectures on Question Formulation.
3. The two day staff seminar and subsequent staff study project on "The Impact of Long-Term Hospitalization on the Family Unit."

1. The Casework Workshop was initiated for new staff members to provide an opportunity to concentrate on problems of psychosocial diagnosis in a clinical research setting. The workshop leader is a member of the staff assigned to the National Institute of Mental Health. She was formerly a Professor of Social Casework at the University of Connecticut. These discussions proved to be of such interest that they have been continued on a voluntary basis. The membership has doubled and the discussions are held bi-weekly throughout the year.

A recent review of the individual case presentations representing each Institute revealed the specialized problems and solutions relevant to cases at the Clinical Center. For example, there is a repeated emphasis on problems of dependency associated with long-term illness, and on problems created by the imminence of death as a result of incurable disease and/or surgery. At the present time, the material is being compiled for use as the focus of a seminar for total staff, and as a further study project.

2. The Research Orientation Lectures on Question Formulation

were developed in order to provide effective social work participation on interdisciplinary research projects. It was considered essential for staff members to be familiar with certain basic concepts of the research method.

Although the Master's Program in Schools of Social Work requires a course in research methods and an experience in research as a partial fulfillment for the degree; the program emphasis, until very recently, has been on the acquisition of social work knowledge.

In order to provide the most appropriate orientation for social work participation in a clinical research setting, the total staff and eleven professors of social work research in Schools of Social Work were polled to determine the most suitable material for orientation lectures.

The consensus was the need for help in the very basic problem of question formulation.

(In this connection, it may be of interest to note that "a survey of a selected sample of members of the Social Work Research Section, National Association of Social Workers, indicated that social work practitioners participating in research either function or are expected to function in almost all aspects of research. However, the tasks most frequently performed by, or expected of, practitioners are those clearly related to the practitioner's knowledge and skill in his own method. Of these the one performed by far the least adequately has been the defining of problems of practice for research.")[1]

At the request of the department, Dr. John Clausen, Chief, Laboratory of Socio-Environmental Studies, NIMH, willingly agreed to give a series of eight informal lectures, using illustrative exploratory and confirmatory studies from the Laboratory as well as related social work material.

The lecture series is now being compiled into a work manual for reference by present and future staff members in their research participation in the various Institutes.

---

[1] Mencher, Samuel, The Research Method in Social Work Education Council of Social Work Education, New York 1959, (Based on response of 46 research specialists selected from members of the Social Work Research Section, NASW, who have had contact with social work practitioners in research projects.)

3. Experience in the Clinical Center has reinforced the total staff's concern with the impact of chronic illness on the family constellation. The opportunity which the Clinical Center provides for knowing members of chronic patients' families has sharpened staff's awareness of the effect of the chronic illness of one family member on each member of the family constellation. The department was most interested in increasing its understanding of the nature of the impact of chronic illness and its effect on family life. It was the conviction of the staff that deepened awareness can promote greater sensitivity to imminent breakdown in family functioning, so that intervention can be initiated on a preventive basis.

Miss Eleanor Cockerill and Mr. Werner Lutz, Professors of Social Work, University of Pittsburgh, who have given leadership in further understanding of the interaction of family roles in illness, accepted an invitation to lead a two day staff seminar on "Family Diagnosis in Chronic Illness."

As a follow-up to this stimulating experience, a staff study project was initiated on "The Impact of Long-Term Hospitalization on the Family Unit."

A preliminary survey was conducted for the total adult patient population for the week of May 11, in order to determine the nature of familial relationships in our patient group. At the time, there was an impression among a number of staff members that a majority of patients were separated from their families or were unattached individuals.

The findings of this preliminary survey indicated that over two-thirds of the 370 patients tabulated were presently members of a family unit. Secondly, that two-thirds of the patients' families fell within the category "Easily Accessible" -- that is, that a family member can be interviewed during the social workers' workday.

In October 1959, the exploratory phase of the project was begun. Sixteen patients (who had been or would be in the Center 30 days or longer) and their available family members were interviewed to learn the effects of a long-term hospitalization experience on certain phases of family social functioning relevant to social work planning.

A review of this data revealed the following significant material warranting further exploration and study:

1. The repeated observation that the patient tends, sometimes at great personal cost, to attempt to maintain in the hospital the role in the family he considered to be his most significant contribution to family life.

2. A chronic illness is present in at least one other family member in the majority of the cases in the study.

3. The loss of the companionship of the hospitalized member was noted as an important concern on the part of the family member remaining at home.

4. A group of cases wherein the wife and/or husband displayed a lifetime of intense devotion to the patient, frequently to the exclusion of all other interest, suggested the need to explore for the presence of this pattern in marriages in chronic illnesses, and their significance both as a source of assistance to the patient, and a source of resistance to resuming a more productive life.

The later phases of the study will concentrate on the most significant areas for social work planning, and will be considerably enlarged in terms of numbers of patients.

The Extramural Program

As a part of the extramural program, two members of the staff have had the opportunity to attend a two weeks' seminar on Casework Treatment for Children at the Smith College School for Social Work. This is an area of special concern at the Clinical Center. Their lecture notes and comments were reported to total staff.

The Program Supervisor in the National Institute of Neurological Diseases and Blindness is presently enrolled in the doctoral program of the University of Minnesota. His training is being sponsored by the U.S.P.H.S. under the Training Outside the Service Program. His majors are research and public health.

During the past year, two former staff members have completed doctoral programs at the University of Minnesota and the George Warren Brown School of Washington University. They have returned to staff positions as a group worker in the National Institute of Arthritis and Metabolic Diseases, and as a caseworker in the Endocrinology unit of the National Cancer Institute.

Progress

A new telephone system was installed to provide secretarial coverage both at the Central Office and Institute level. It assures reception of all incoming calls.

Because the volume of patient control cards has become excessive with six years of operating experience, the assistance of the Management Analysis Section, O.D., has been requested to review present procedures and recommend more economical ones for future use.

Since several staff members have participated in closed circuit T.V. case presentations, the department is exploring the possibility of using these video-tapes for demonstration, recruitment, or exhibit purposes.

Recommendations:

1. To continue to provide adequate social work coverage to all patients and their family members as required.

2. To continue to encourage the interest and participation of staff members in areas of research where their training and experience can be of value.

3. To enrich and deepen staff members' knowledge of research methodology, so their participation in research will be meaningful and appropriate.

4. To review present classification and salary levels of positions in this department, in relation to the new Civil Service standards for clinical social work positions. These standards greatly reduce the amount of experience required for the various grade levels, i.e., we currently require four years experience after the master's degree for a GS-10 position while the new standards require only one year of experience

Honors, Awards and Publications

Dr. O'Keefe	Elected Chairman, Psychiatric Social Work Section, National Association of Social Workers, 1959-1961. Member, Medical Social Work Advisory Committee, American Cancer Society, 1959-1960. Editor, Proceedings of Third Annual Work Conference on Psychiatric Social Work, "The Psychiatric Social Worker as Team Member and Consultant," sponsored by The School of Social Welfare, Louisiana State University and the Louisiana State Department of Hospitals, Baton Rouge, Louisiana. Institute Leader, Ohio Welfare Conference, "Administration of Social Service Departments in Multidisciplinary Settings." "Psychiatric Social Work," for Social Work Year Book, 1960.
Miss Walsh	Member, National Practice Committee, Medical Social Work Section, National Association of Social Workers, 1959-1961. Representative, Medical Social Work Section, to the Planning Sessions for meetings on Staff Development to be held at the National Conference of Social Work, February-April, 1959. Visiting Lecturer, American Red Cross National Training Center, Charlottesville, Virginia, to lecture in March and October on "The Meaning of Illness to the Patient and his Family." Member, Public Relations Committee, Washington Chapter, National Association of Social Workers.
Mrs. Basamania	Presented paper, "Toward a Family Diagnosis and Treatment of the Family Unit," and participated in day long workshop at the Annual Meeting of American Orthopsychiatry, April 1959, in San Francisco. The paper has been accepted by the American Journal of Orthopsychiatry.

Honors. Awards and Publications: Continued

Mrs. Basamania	Publication of paper co-authored with two psychiatrists. "The Role of the Father in Families with a Schizophrenic Patient." American Journal of Psychiatry, May 1959.
	Author of paper, "Casework Study and Treatment of the Mother of Quadruplets each with a Diagnosis of Schizophrenia." Paper submitted to the Program Committee of the American Orthopsychiatric Conference.
Mrs. Berkman	Attended the National conference of the National Association of Social Workers held in San Francisco the week of May 25-29, 1959.
Miss Carter	Served as a member of the Personnel Practices Committee of the National Association of Social Workers which is currently working on a revision of the code of ethics.
Mrs. Dowling	Elected Chairman of the Metropolitan Washington Chapter, National Association of Social Workers, taking office July 1, 1959 to continue until July 1, 1961.
Miss Fugitt	Member, Executive Committee of the Medical Social Work Section of the Metropolitan Washington Chapter of the National Association of Social Workers.
	Member, Social Work Committee for Professional Education, D. C. Division, American Cancer Society. This Committee in conjunction with the Nurse Committee, sponsored a Symposium on "The Cancer Patient in our Community," May 7, 1959, at the Washington Hospital Center. As a member of the panel, Miss Fugitt presented a paper.
Mr. Hirsch	Presented a paper before the Maryland Chapter of the National Association of Social Workers.
Mrs. Kilian	Participates regularly in the twice-monthly meetings of the National Cancer Institute Clinical Chiefs at the invitation of the Clinical Director
	Participates as a member of the Intake Committee of the Health and Welfare Council held monthly.

Honors, Awards and Publications: continued

Mr. Mabon	Discussion leader at elementary school P.T.A. meetings. Member, Committee on Rehabilitation Resources of the Greater Metropolitan Area, sponsored by the Washington (D.C.) Medical Society. Director of Montgomery County Rehabilitation Center for discharged mental patients.
Mrs. Murphey	Presented the College Study Project before the Annual Meeting of the American College Health Association.
Mr. Nye	Gives weekly orientation lectures to the Central Nursing Department.
Miss Peay	Attended the American Psychiatric Association, Philadelphia, Pennsylvania, April 29 and 30, 1959. Member, Program Committee, Medical Social Work Section of the National Association of Social Workers. Member, Social Service Staff Research Evaluation Committee. Member, Reprint Committee, National Association of Social Workers.
Mrs. Wilkie	Chairman, Psychiatric Social Work Section of the National Association of Social Workers, D. C. Chapter. Member, Executive Committee of the Maryland Chapter of the National Association of Social Workers. Chairman of the Educational Committee, National Association of Social Workers, D. C. Chapter. Member, Board of Directors of the Montgomery County Mental Health Association.

Honors, Awards and Publications: Continued

Mrs. Wilkie	Chairman, Speaker's Bureau, Montgomery County Mental Health Association.
	Presented to the Montgomery County Mental Health Association on Marital Relations.
	Presented before church groups, P.T.A. groups, parent groups, etc., on mental health aspects of childhood.
Staff	Attended the two day meeting at Saint Elizabeths Hospital under the auspices of Saint Elizabeths Medical Society.
	Attended the Washington School of Psychiatry lecture and film given by Dr. Nathan Ackerman on the "Psychodynamics of the Family."
	Attended the Psychology Institute at the National Institutes of Health.
	Attended the National Conference of the Family Service Association of America.

[illegible] I

[illegible]

[illegible]

[illegible] NO. II

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	25	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[illegible]

III

IV

Calendar Year 1959

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT
CLINICAL CENTER

CC-34
Serial No.

ANESTHESIOLOGY DEPARTMENT

The Anesthesiology Department carried out its primary objectives of providing anesthesiological care for all patients undergoing therapeutic surgery, or diagnostic procedures requiring anesthesia, and for the conduct of the various types of inhalation therapy for all Clinical Center patients. Research activities were limited to a number of clinical projects in which newer anesthetic agents and methods were employed as a means of improving the overall care of the patient. Direct collaboration with investigators of several of the institutes was done in projects primarily concerned with use of anesthetic drugs. Lack of research personnel precluded laboratory investigations during the past year.

Our full-time staff was composed of the following personnel (based on the yearly average):

Clinical Anesthesiologists. .	4
Research Anesthesiologists . .	0
Nurse Anesthetists	3
Inhalation Therapists	3
*Anesthesia Technicians . . .	2
Secretary	1

The services of outside consultant anesthesiologists were utilized three or four days per week on a per diem basis, to supplement our regular staff.

I. ANESTHESIOLOGICAL PROCEDURES. The administration of anesthesia was done in 1,281 instances; surgical operations accounted for 863, and diagnostic procedures under anesthesia were carried out 418 times. Ninety-seven additional related procedures were done, including resuscitation measures outside the operating room in 29 instances.

As in previous years, a considerable proportion of the operations performed at the Clinical Center were rather lengthy.

Anesthesia time exceeded 3 hours in 504 operations (41%)
Anesthesia time exceeded 5 hours in 229 operations (18%)
Anesthesia time exceeded 6 hours in 124 operations (9.6%)

*Assigned from the Surgical Nursing Service, last half of year.

Anesthesiology Department

Extensive excision surgery for the treatment of cancer; surgical correction of cardiac defects with the aid of extracorporeal circulation; localization and extirpation of epileptic foci in the brain, and removal of intracranial tumors with the aid of hypothermia, account for a large part of our work. Extracorporeal procedures, during which the heart and lungs were bypassed, were done in 104 instances. Hypothermia was utilized in the treatment of 18 patients, 13 during neurosurgery. Hypothermia was used in cardiac surgery only on 3 occasions. There was a definite trend away from the use of lowered body temperatures, in favor of extracorporeal circulation technique in the performance of cardiac surgery. The high preponderance of complex operations made it imperative that two trained anesthetists be available for the management of patients during lengthy procedures, and that adequate technical assistance be available for the setting up and operation of anesthetising apparatus, respiratory ventilators and various monitoring devices.

Diagnostic studies done under anesthesia form a large part of our work. A total of 418 anesthesias (32.6%) were administered for this purpose, the majority in locations outside the Main Surgical Suite. Some diagnostic procedures, particularly cardiac catheterization studies, required three or more hours of controlled sedation with anesthetic drugs to permit their completion.

The table below indicates the institutes responsible for the surgical cases with whom we worked. Four of the seven institutes have surgical staffs, the majority of surgery being done by the first four institutes listed below:

Administration of Anesthesia, 1959

Sponsoring Institute	Procedures	Percent
NHI	* 503	39%
NCI	278	22%
NINDB	270	21%
NIDR	137	11%
NIAMD	39	3%
NIAID	52	4%
NIMH	2	0.1%
	1,281	100%

* 222 of this number were for diagnostic studies.

II. INHALATION THERAPY SECTION ACTIVITIES. The following types of inhalation therapy treatments were carried out with the assistance of three inhalation therapists. The approximate number of hours are also indicated. These treatments were as follows:

Oxygen tent therapy	6,984	hours
High humidity tent therapy	12,741	hours
Incubator with high humidity	2,001	hours
Hydrojette (cold steam)	2,254	hours
Oxygen administration by catheter or mask	7,338	hours
Aerosol therapy	5,374	treatments
Intermittent positive pressure breathing with aerosols	2,322	treatments
Respirator use, long term	590	hours
Resuscitator (artificial respiration)	409	hours
Administration of oxygen combined with other gases	550	hours

A teaching program for the orientation of new personnel in the use of standard oxygen and suction devices employed in the Clinical Center, was carried out through weekly demonstration sessions. A total of 138 registered nurses, 26 practical nurses, 42 nursing assistants, and 6 other nursing personnel attended these sessions. On seven occasions, special demonstrations of specific respiratory equipment and motion picture films depicting methods of artificial respiration were arranged for interested groups of physicians and nurses.

III. RESEARCH ACTIVITIES. We had no research anesthesiologists on our staff during 1959. We did collaborate in a number of projects.

Dr. G. R. Christenson served as one of two principal investigators in the project sponsored by NIDR, which involves recording and study of the physiologic responses of ambulatory dental patients during dental surgery under general anesthesia. Parallel studies were made of the effects of hexocyclium, a new drug for the control of secretions during oral surgery and the anesthetic properties of methohexital, a new, short-acting barbiturate which may prove superior to thiopental for dental anesthesia. During the last part of 1959, physiologic monitoring of vital functions was made more complete by the addition of an ear oximeter to measure the oxygen saturation of arterial blood during the actual performance of dental surgery. This particular project required the services of two anesthetists and one anesthesia technician, one-half day each week, in addition to the services of the dental team. The project description is being submitted by the principal investigators, Dr. Christenson and Dr. Driscoll of NIDR.

Anesthesiology Department

Measurement of the effects of various drugs on myocardial contractility were made during cardiac surgery by means of a strain gauge sutured directly to the heart muscle. We utilized this opportunity to study the effects of a number of anesthetic agents and muscle relaxants. Dr. R. C. Brown and Dr. G. R. Christenson of our staff are collaborating with Drs. Goldberg and Bloodwell of NHI on this project, which will be reported by NHI.

In a search for an anesthetic agent which could be used for the preliminary opening stage of exploratory craniotomies in epileptic patients, without affecting the electroencephalogram recordings from the cerebral cortex later in the operation, Dr. G. R. Christenson has begun the study of methohexital for this purpose. Preliminary trials have been encouraging.

IV. MAJOR PROBLEMS ENCOUNTERED. There were no new problems during 1959. During the first half of the year we were again confronted with a severe shortage of anesthesiologists to carry out our heavy surgical schedules. The shortage was met by increasing the number of visits from our consultant anesthesiologists, and by rearranging the scheduling of operations to meet the supply of anesthetist personnel. Recruiting efforts exerted during the past two years resulted in the addition of two trained anesthesiologists to our group in July 1959, giving us an adequate number of physician anesthetists to handle the current workload.

The procurement of anesthesiologists, under provisions of the Commissioned Officers Residency Deferment Program, is almost the only means at our disposal for the recruitment of future staff members. During the past year, we have continued to seek out prospective anesthesiologists, to interest them in enrolling under the CORD Program for future assignment to the Clinical Center. In this way we have assured ourselves of replacements for the two year periods beginning July 1960 and July 1961.

We have found it increasingly difficult to locate skilled, well trained nurse anesthetists to work as members of our anesthesia teams. Apparently, Civil Service pay scales do not attract the high calibre nurse anesthetists we require. We have had considerable difficulty in finding replacements for inhalation therapists. We have not been able to find candidates with the intellectual and personality characteristics necessary for this position. The available career opportunities and rate of compensation have not enabled us to: (1) recruit and train suitable candidates, or (2) attract personnel trained at other hospital centers to come to this locality.

We have also experienced a problem in trying to find suitable technician personnel to work as part of the anesthesia team during prolonged operations. The operation and maintenance of anesthetic apparatus and monitoring devices calls for special knowledge and training, which should rate somewhat higher than the GS-3 classification. We are looking for some way by which this difficulty can be overcome.

During the later part of the year, certain problems arose following the establishment of new restrictions concerning apparel to be worn in the operating rooms of NHI, as a means of reducing air borne infection hazards. Difficulties were encountered in maintaining communications, procurement of blood and in obtaining outside assistance. The existence of two separate sets of working regulations for the operating rooms used by NHI and NINDB called for the utmost in cooperation by anesthetist personnel.

V. CHANGES AND IMPROVEMENTS. There were no outstanding changes made during the year. There was a definite improvement in our staffing situation with the addition of two anesthesiologists to our full-time staff. It is also noteworthy that the importance of inhalation therapy was recognized by reclassification of our senior inhalation therapist's position to a higher grade. It should also be reported that we have been successful in maintaining an extremely low incidence of serious complications and sequelae attributable to our anesthesia techniques in the management of Clinical Center patients undergoing the most complex types of surgical procedures.

VI. PUBLICATIONS AND PRESENTATIONS.

1. Driscoll, E. J., Christenson, G. R., and White, C. L. Physiological Studies in General Anesthesia on Ambulatory Dental Outpatients. To appear in Journal of Oral Surgery, Oral Medicine and Oral Pathology, December 1959.

2. Hebert, C. L. Monitoring Devices as Aids in Anesthesia. Presented at 19th Annual Conference Tri-State Hospital Association, Washington, D. C., October 26, 1959.

3. Hebert, C. L. Management of Anesthesia and General Hypothermia During Neurosurgery. Presented at Combined Clinical Center Staff Meeting, October 8, 1959, as part of symposium. Scheduled for publication in Annals of Internal Medicine.

Anesthesiology Department

Staff members attended the following national meetings:

1. Institute for Nurse Anesthetists, San Francisco January 26-30, 1959 (Miss Joyce)

2. Congress of International Anesthesia Reseach Society, Miami Beach, Florida, April 19-23, 1959 (Dr. Hebert)

3. American Society of Anesthesiologists, Annual Meeting, Miami Beach, Florida, October 5-9, 1959 (Dr. Christenson)

4. Annual Anesthesiology Review Session for the Armed Forces, Lackland Air Force Base, Texas, June 7-13, 1959 (Dr. Christenson)

5. Postgraduate Assembly in Anesthesiology, New York, December 9-12, 1959 (Dr. Dean Morrow)

6. Dr. Hebert served as associate examiner for examinations held by American Board of Anesthesiology, Hartford, Conn., September 27-October 3, 1959

C. L. Hebert

C. L. Hebert, M. D.

Calendar Year 1959

PUBLIC HEALTH SERVICE   NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

DIAGNOSTIC X-RAY DEPARTMENT   CC-35
Serial No.

Program Goals:

The primary objective of the Diagnostic X-ray Department is to provide a complete diagnostic radiological service for the seven institutes of the National Institutes of Health. Other objectives include the operation of an isotope clinic providing diagnostic brain scans and thyroid uptake examinations; operation of X-ray facilities for animal research; and the provision of consultation on radiological problems.

Developments and Trends:

In accordance with new Board requirements for certification in Radiology, a four year residency training program has been inaugurated and the necessary clearances obtained. This program will become effective in July 1960.

Consistent with new film processing developments, a Kodak X-Omat film processing unit was placed on order during calendar year 1959. This unit will permit procurement of dried films in seven minutes and will greatly facilitate operation of the X-ray department.

Due to the development of an unusual number of infections in patients following cardiac surgery, more stringent sterile techniques were inaugurated in the Diagnostic X-ray Department for heart patients undergoing angiocardiographic studies. This was an added precautionary measure, although there were no indications that these patients contracted their infections within the X-ray department.

Several new contrast media were investigated, in keeping with the trend toward steady development of new opaque media and the improvement of diagnostic methods. The most effective of the newer media are utilized by the department for contrast examinations.

The Diagnostic X-ray Department assumed the responsibility for performance of brain scans using I 131. The assumption of this function, plus the appreciable increase in the number of thyroid uptake studies performed within the department, required more technical personnel time than previously anticipated. A full time employee will soon be necessary to meet this responsibility.

A series of new conferences were undertaken by the Diagnostic X-ray Department during calendar year 1959. These conferences are directed primarily toward instruction of Clinical Center physicians. This development is an outgrowth of requests made by various clinical associates.

Plans and Future Work:

Due to the marked improvement in fluorescent image intensification and cineradiographic techniques, immediate plans call for procurement of equipment of this type to meet the responsibilities of the X-ray department in providing the most up to date methods available. These new developments in X-ray equipment permit more accurate and prompt X-ray diagnoses.

A resident in radiology will be assigned to immediate viewing of all films processed by the new Kodak X-Omat. This will facilitate rapid reporting, as well as the procurement of films of suitable technical quality. It is anticipated that the number of patient recalls to the department due to unsatisfactory film will be significantly reduced by the new procedure.

New isotope equipment has been requested and should be delivered during calendar year 1960. With this new equipment, it will be possible to reduce the radiation dosage to the patient by at least 50 per cent. In addition, the thyroid uptake studies should be more accurate since the new equipment will be more effective in reducing the background count.

Plans call for the addition of a staff member to the Diagnostic X-ray Department for assumption of responsibility for performance of surgical procedures in conjunction with diagnostic radiographic procedures. This staff physician will be responsible for teamwork on angiocardiographic studies, for supervision and actual performance of cerebroarteriograms, pneumoencephalograms, myelograms, etc. It is believed the addition of a person of this type will be of great benefit to the medical care program at the Clinical Center, as an experienced person versed in all phases of these examinations will be able to reduce some of the complications now encountered in the performance of these special procedures.

The ordering of radiographic equipment for the new surgical wing, and the procurement of trained personnel to operate these radiological facilities, will be the responsibility of the X-ray department. Preliminary plans have been drawn up, but the constant improvement in and development of new radiographic equipment necessitates a delay in placing of the final order until the latest date practical.

As in previous years, every effort will be made to provide the best diagnostic radiologic service in the world. This will require financial support to obtain the required equipment and personnel.

Progress Achieved:

There was an increase of approximately 15 per cent in the number of radiographic studies performed during fiscal year 1959 as compared to 1958. The number of studies performed for the two fiscal years were 33,606 and 28,794 respectively. There was also an increase of 42 in the number of special surgical procedures performed within the department. In addition, 518 thyroid uptake studies and 41 brain scans were consummated during the fiscal year.

After two years of concerted effort, the promotion of the Chief X-ray Technician to a GS-11 grade was accomplished. A Career Officer was also added to the staff of the department as a radiologist and subsequently promoted to Assistant Chief of the Department.

A joint research study on the intraocular vascularity of the eyes in animals was consummated with NINDB and the Diagnostic X-ray Department. This study led to the constuction of an exhibit on the same subject which was very well received at a national radiological meeting.

An order was placed for the purchase of a Kodak X-Omat processing unit. Likewise the necessary clearances were obtained from the Plant Engineering Branch to provide the power and water supply needed for rapid installation of the unit upon its receipt. This is essential in order to expedite installation and avoid impeding the functions of the X-ray department.

Papers were presented at the International Congress of Radiology, at the North American Radiological Society, and at the Eastern Conference of Radiologists. In addition, many lectures, X-ray conferences, and teaching functions were conducted by the department. Consultation was also provided to other government agencies and Public Health Service organizations upon request.

There was a decided increase in the thyroid uptake studies performed in comparison with previous years. There was also a significant increase in the number of special procedures performed. This is a reflection of the increase in service functions required of the department.

Major Problems Encountered:

The major problem of the department during calendar year 1959 continued to be that of procuring and retaining skilled technical personnel. Salary inducements outside the Service resulted in loss of key technical personnel. The delays in employment procedures secondary to Civil Service regulations, and the apparent increasing shortage of X-ray technicians further complicated recruitment difficulties. The only solution to this problem appears to be the establishment of a technician training school within the Clinical Center.

The number of mechanical and electronic breakdowns on radiographic equipment increased during calendar year 1959, as compared to all previous years. This, of course, is to be expected in view of the fact that the equipment is now approximately six and one half years old. Some components of X-ray equipment may last for years, but the budgeted life expectancy of X-ray equipment is normally eight years.

The necessity of adequate financial support to replace and renovate worn out equipment became more apparent during 1959. This, of course, is inherent in the increase in the number of equipment breakdowns. A realistic approach to this problem will require a continuing budgeting of funds to replace equipment, and to install newer and improved radiographic equipment if the department is to remain an up to date, modern radiographic service.

Other problems of a less serious nature include inaccuracy in isotope calibration necessitating a change in supplier of I 131; the occurrence of a series of bizarre artefacts on X-ray films which was resolved by demanding more careful handling of films during shipment; and the development of a technical problem with the Schonander head unit which was alleviated after considerable study by changing the type of bucky grid.

One problem which remains unresolved at present is that of storage of films on old patients in the inactive category. With the increasing patient load, and particularly with the more frequent return of old patients, the use of these old films and their return to the active files has increased steadily. The storage area currently available for these films is in the basement at the far end of the Clinical Center. It is hoped more suitable space may be obtained to alleviate the considerable waste of time in film transportation.

Honors, Awards, and Publications:

The Chief of the Department was invited to present a paper at the International Congress of Radiology in Munich; at the North American Radiological Society in Chicago; and at the Eastern Conference of Radiologists in Washington, D.C. The papers presented were well received.

An exhibit on "Experimental Intraocular Venography," prepared by Dr. Cohen of NINDB and Mr. Morel of the Diagnostic X-ray Department, won a bronze medal for excellence at the American Roentgen Ray Society meeting in Cincinnati. Another exhibit entitled "Roentgen Complications of Selective Angiocardiography", presented at the North American Radiological Society meeting in Chicago, also received special recognition and a letter of commendation.

The Department Chief was appointed a member of the Program Committee of the Eastern Conference of Radiologists, and also a member of the Radiation Committee of the District Medical Society.

There were four papers published or accepted for publication by staff members during calendar year 1959. Papers were also presented by staff members to the Johns Hopkins Alumni Association; the Eastern Conference of Radiologists; at Walter Reed Army Hospital, Johns Hopkins Hospital; and at Georgetown University. These papers were not submitted for publication. In addition, a number of lectures were given by departmental staff members at various meetings held at the Clinical Center.

PUBLIC HEALTH SERVICE — NATIONAL INSTITUTES OF HEALTH

[illegible] ANNUAL REPORT OF PROGRAM ACTIVITIES: CLINICAL CENTER

DENTAL DEPARTMENT

As has been stated in previous annual reports, the objective of the Dental Department of the Clinical Center is to provide a diagnostic and treatment service for the various Institutes of the National Institutes of Health. The aim of the Dental Department is to perform a complete dental examination on every patient admitted to the Clinical Center. Recommendations for treatment are made to the attending physician and dental treatment is rendered to those patients for whom it is requested.

In previous annual reports, a number of examples of cases which make the work of the Dental Department somewhat more difficult than that of other similar facilities, have been cited. We still have the problem of the mental patient who needs additional appointments to gain his trust and confidence. We also have unusual problems with hemophilic cases and with patients with low I.Q. There are quite a number of more unusual types of procedures in which we cooperate with various Institutes for the proper solving of the problem.

We have assigned one staff dentist to cooperate with the National Cancer Institute in the handling of leukemias. He is attending Ward Rounds regularly and is participating in the daily care to prevent debilitating oral syndromes. During the past year, a paper has been submitted for publication, "Thrush in Malignant Neoplastic Disease"

Stress is being placed on proper dental care before radiation of oral tissues. Extraction of teeth, in the line of radiation before treatment is begun, is insisted upon. Pre-operative wiring of teeth, in surgical cases which involve the mandible, is routine. The practice of constructing pre-operative splints for maxillary surgery was originated in our Clinic. A new type of soft obturator, for replacement of missing parts of the palate, was tried. The results were reported at a meeting of the Academy of Maxillo-facial Prosthetics, in New York. Considerable success has been had in the proper handling of extractions of teeth for hemophiliacs. These operations require much more than ordinary care; so routine splints were constructed and the patients were well controlled by these local measures along with the administration of fresh plasma. In relation to our work with the National Institute of Mental Health, we are in receipt of a memorandum from Dr. Philip U. Spielman, Assistant Chief, Children's Service. It is as follows:

"I want to thank you and your staff for your cooperation in the treatment of our patients. Not only did you graciously provide us with the many special appointments and emergency help we needed, but you were also able to patiently and constructively work with these emotionally disturbed children.

"Initially these children greatly feared dental procedures. As a result of the efforts of your staff the children were helped to master many of their fears and anxieties about dental care. Eventually some children even came to look forward to, or even to demand, visits to the Clinic. In addition to serving the children well during their hospitalization period (which is now ended) you also provided them with experiences that will help them in the future. Those who will be responsible for their later dental care will most certainly profit from your work."

The National Institute of Dental Research has an excellent study on Human Genetics, [illegible] Institute [illegible]. In order to facilitate the investigations, a number of cases which had problems were submitted to the Dental Department of the Medical Centre for solution. Three examples can be given: A seven year old boy with amelogenesis imperfecta with teeth eroded down to the gum tissue. Some of the sharp edges of the teeth were rounded and full crowns were [illegible] over the ridges with the fragments and tooth remnants still in place. In reference to the second case, we are in receipt of a letter from Dr. Lawrence L. Frost, Director, Child Guidance Clinic of the Juvenile Court of the District of Columbia, who writes:

"On behalf of Judge Ketcham, Cecil [illegible], and myself, may I express sincere thanks for what you and your staff have done for Cecil.

"The event in Court [illegible] this afternoon and we saw a genuine smile for the first time since we have known him.

"We have no knowledge that this will keep Cecil out of trouble, nor do we harbor any illusions that what you have done for him will alter his personality in some miraculous fashion. But we do know that through your interest and help, Cecil has been given an opportunity to make something of his life."

The third case is another of amelogenesis imperfecta in a 12 year old boy whose teeth were all eroding away at a rapid rate and this made it necessary to put full crowns on every tooth in this patient's mouth, which is a very extensive job of oral rehabilitation. He is a very bright boy and his ambition is to become a medical missionary.

There has not been an increase, over previous years, in the number of examinations performed, total number of visits and the total number of treatments. We have apparently reached a plateau. It is possible that another explanation is that we had an unusual amount of sickness in the professional staff during the past year. It has also been noted that the various institutes are not referring quite as many patients for examination.

It is becoming more apparent that we will need an additional dental technician. The pattern of work performed in the laboratory is such that we will, without question, need to expand this facility. This can only be done by hiring a dental technician. During the past year, we were able to reclassify the jobs for the technicians to a more equitable pay scale. This has improved the situation because we were in danger of losing these men to outside opportunities. The auxiliary personnel of the dental staff is quite satisfactory. We again state, however, that it probably will be necessary to obtain the services of the additional girl in the near future.

A proposal has been made to establish a Department of Radiology in the Dental Department. Need has been demonstrated for someone to take charge of this activity to expand its purpose and to improve our service. Present plans call for this job to be placed in the National Institute of Dental Research staffing pattern. The person in charge of the Department of Radiology will be principally responsible for cephalometric X-rays, their tracing, and for the more unusual X-ray procedures. He will have supervision of a dental assistant assigned to take routine X-rays of the patients for the Dental Department.

Calendar Year 1959

PUBLIC HEALTH SERVICE   NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

CLINICAL PATHOLOGY DEPARTMENT

CC-37
Serial No.

Introduction

Because of the change of the annual reporting period to that coinciding with the fiscal year, this report overlaps the last six months (July-December 1958) of the previous annual report. Therefore, statistics for the past fiscal year are not independent of the previous annual report. To clarify trends we will compare the unreported six month period (January-June 1959) with the same period of the previous year (January-June 1958), thus eliminating statistical overlap, and excluding the effects of clinical staff changes, new projects, etc., which would influence comparison of the first half of calendar year 1959 with the last half of 1958.

Therefore, this report is based primarily on the six months beginning January 1959.

Mission

The Clinical Pathology Department is organized to provide laboratory consultation and services of research quality in the areas of hematology, clinical chemistry, microbiology and special diagnostic procedures. One of our most important obligations is the application of methods from the rapidly advancing fields of basic sciences and research in medicine to serve the purposes of daily patient care. Growth of work in this phase will continue to require more attention and often time-consuming meticulous operations. These efforts not only widen the scope of significant analytic information, but frequently yield more economic, accurate and rapid technics, thereby increasing our capabilities. Another basic responsibility is the development of a comprehensive but efficient system of records, reports and information processing for laboratory data. An enormous potential for important research in correlating chemical, metabolic, physiological and immunological patterns of individuals and establishing individual norms is being lost because of our (meaning all clinical laboratories) ineffective and limited record methods.

Workload (Figures based on period January-June 1959)

An average census of 401 patients in seven Institutes resulted in 51,438 patient workdays during this six month period. These patients required 180,708 test procedures, an increase of 19% (29,397 tests) over the similar six month period in 1958. As predicted in the previous report, the average monthly test load has increased to 30,000 where it appears to have leveled off under present conditions. However, the additional requirements of expanding clinical investigations and new

laboratory procedures will increase this number somewhat each year. (Total statistics for calendar year 1959 are listed in the appended tables).

During this period the average technical staff for each working day decreased from 38.1 to 37.7. This decrease in staff and the 19% increase in test procedures resulted in a further increase of tests per technologist per day from 31.4 to 39.3 or a 25% increase. Furthermore, an intangible increase of pressure is brought about by a higher proportion of more complex and time-consuming procedures requiring meticulous operation.

Despite some periods of discouraging workload we have succeeded in maintaining reliability and accuracy of research quality by untiring effort, loyalty and overtime work by our entire staff and by applying modern laboratory developments, especially mechanization. The adoption of automation, special methods and equipment has increased each technologist's daily productivity. A resulting increase of the maximum individual load compatible with optimum efficiency and accuracy from 25 to 28 tests per technologist per day was attained, although during the period of this report, we were required to average 39 tests per day per technologist or 39% above the optimum efficient load. We can expect further automation to increase optimum performance to 30 tests per day and later probably 35.

Quality Control

Quality controls have been extended to as many procedures in microbiology as can benefit from available control measures. All our staff is now alert to and thoroughly indoctrinated in quality control concepts and methods. We find that our technologists take professional pride in maintaining their control charts and in constant surveillance of the reliability of their work. The control charts permit confidence in methods and instruments and assurance that defects of operation will be immediately apparent. Patient care physicians have learned the same confidence and although the evidence is intangible, there are almost no instances of repeat requests to check the reliability of a test report. This effectively reduces unnecessary work in our department.

Personnel

The new standards for classification of technicians and technologists (professional grade) issued by Civil Service were finally put into effect and our positions and position descriptions rewritten. Qualified technologists were rerated to conform to the new standards which provided GS-9 professional grade positions for our laboratory supervisors. Recruiting of qualified technical staff immediately improved. Although the actual increase in technical staff was only an average of 6 over the previous year and did not fill our established positions, nevertheless, the relief of work pressure immediately reduced overtime and greatly lifted morale. For the past five months we have managed to complete work and transmit reports by 5 on most workdays.

Dr. Elizabeth Frame, Chief, Clinical Chemistry Service, resigned June 30th to transfer to the Research Grants Branch. Dr. Frederick Fox, who completed his residency on June 30th, was appointed Acting Chief of Clinical Chemistry starting July 1st. He has done a superb job in maintaining the high level of accurate and reliable work in his Service, and in maintaining morale and progressive training of the staff.

Dr. Gunnar Jungner, Professor of Clinical Chemistry, University of Gothenberg, joined our Clinical Chemistry Service on October 1st, as Visiting Scientist. He has already contributed to the professional prestige of our Clinical Chemistry Service, and participates actively in all our work and training programs. He brings to us a wide and profound knowledge of clinical chemistry and many excellent ideas from a long practical experience.

Dr. J. J. Huang, Visiting Scientist in Microbiology, terminated his visit in February 1959 to return to WHO, somewhat earlier than anticipated, but he found it necessary for his future to accept an offer of tenure we could not match. Dr. Edna Schneper returned to our Microbiology Service part time as Acting Chief on July 1st.

Dr. James Peden resigned from Hematology to return to Washington University School of Medicine, and was replaced by Dr. J. Russell Little, as Assistant Hematologist.

Dr. H. H. Marsh remains for a third year of experience in clinical pathology, and Dr. Hugo Pribor transferred to Bowman Gray School of Medicine. Drs. Robert Fouty and Rowan Boylan joined our resident staff on July 1st.

Examples of Special Contributions to Patient Care

For Clinical Pathology we have recruited and trained a selected technical staff able to perform research quality procedures and who, in addition to their technical skills, are alert and capable of observations which contribute to the clinical diagnosis or have research implications.

In the past year, an astute observation by a technologist in the Microbiology Service called attention to possible contamination of the heart pump. Subsequent investigation by our group in cooperation with the Surgery staff and Sanitation Section revealed other problems of sepsis in the operating rooms, and recommendations were made for their correction.

Our staff in Microbiology frequently expands the field of requested investigation when findings do not explain the patient's condition. For instance, we discovered two unsuspected cases of histoplasmosis.

We are constantly aware of the Staphylococcal problem and have expanded investigations to include hospital personnel as well as patients. Phage typing remains our most effective means of epidemiological study and is important in the detection of early pre-epidemic foci.

Our Hematology Chief and his staff provide frequent clinical consultation at the request of physicians concerned directly with patient care. The unique method of sequential recording of hematologic determinations provides a record from which minor and major changes are easily detectable. In instances where current daily hematologic data do not coincide with the patient's previous trends, a search for cause is immediately made, commencing with notification of the patient's physician. In many instances, this has led to early diagnosis of a hemorrhagic, infectious, or other medical emergency, the immediate treatment of which frequently has been life saving.

Occasional postoperative bleeding following heart surgery has necessitated technologists remaining on duty for long hours, performing a series of hematologic and coagulation determinations.

Daily observations by the staff of Clinical Chemistry have contributed valuable information to patient care, diagnosis and research. As an example, in the past year a previously unsuspected case of amino aciduria was discovered by the alertness of a technologist during the performance of other determinations.

Clinical Pathology continues to be a vital part of the research effort in all institutes, as demonstrated by our growing participation in the metabolic, heart and cancer services.

Operations

Automation and Specific Test Improvements

During the past year, the Clinical Pathology Department has continued to improve the efficiency of current tests, and to introduce new procedures which will be helpful to the clinical staff in patient-care and clinical research. The technical and economic advantages of automation in the clinical laboratory were pointed out in the Annual Report for 1958. In the succeeding twelve months, the use of automatic instrumentation in Chemistry and Hematology has increased, enabling these services to cope with the rising workload.

The Hematology Service has added several new instruments. A Coulter Electronic Cell Counter was installed in our Follow-Up laboratory on the first floor, obviating the inefficiency of dividing and transporting blood specimens to the fourth floor for counts. Experimentation is in progress on adaptation of a newly modified Coulter instrument (Model B), for counting platelets and obtaining size distribution curves of red cells. The development of an accurate automatic technique for counting platelets would make a most significant contribution to laboratory efficiency and capacity, because it would require less time than counting with phase contrast microscopy.

The Hematology Service is evaluating the Hellige thromboelastograph. This machine automatically measures and records the dynamic physical processes of blood coagulation in vitro. More accurate measurements of these clotting properties should contribute knowledge important in managing patients with obscure bleeding tendency, (cardiac surgery patients, especially).

Research programs were carried on by both Dr. Brecher and Dr. Peden. Contributions were made in the areas of isotopic thymidine labelling of DNA in leukemic white cells, coagulation problems, and the circulation of time of fluorescein-labelled granulocytes.

The Clinical Chemistry Service has introduced several new diagnostic procedures during the past year. The blood ammonia test is useful in the diagnosis of hepatic disease, and particularly hepatic coma. A screening test of 5-hydroxyl indoles have been instituted which allowed the clinician to rule out quickly a diagnosis of malignant carcinoid syndrome.

Adaptation of procedures to automatic instrumentation has been continued. After a control study of 500 duplicate analyses by standard methods, the total carbon dioxide content of serum is now being routinely determined by the Autoanalyzer. This results in a saving of nearly one technologist's time per day. Alkaline phosphatase determinations are likewise being done by the Autoanalyzer. Adaptation of this versatile instrument to the serum glutamic oxalacetic transaminase procedure is being studied. Automation of this test would be of great help, because the present method is long and complex, and there has been a great increase in the number of requests for this test during the past year.

Seligson Automatic pipettes have been introduced into the procedures for phosphorus, thymol turbidity and cephalin-flocculation, which has increased the accuracy and precision of these tests, and shortened the time. Mr. Nishi developed a bivalve for the pipettes which permits rapid repetitive use for delivering as little as 1 ml reagent accurately.

He also modified the amperometric chloridimeter with a second timer further to eliminate necessity of resetting blank level for each specimen. This improvement will be published by him and Dr. Coltove.

Mr. Nishi developed and installed a flow through cuvette for the Evelyn colorimeter which considerably improved its facility. Electrophoresis, phosphorus and alkaline phosphatase procedures have been taken over by the Clinical Chemistry Service, freeing the Control Chemistry Service for more developmental work.

The Control Chemistry Service completed the adaptation of the alkaline phosphatase procedure to the Autoanalyzer. A microglucose determination was also developed for this instrument, which can be done using the same reagents employed for the macroglucose test. The routine blood urea nitrogen and glucose tests have been improved by the addition to the Autoanalyzer of a heated dialyzing unit, and new interference filters which increase the sensitivity of the colorimetric readings. The uric acid and creatinine determinations are in the process of being evaluated and standardized as Autoanalyzer procedures. Evaluation of the inorganic phosphate method published by the manufacturer disclosed marked discrepancies between "dialyzable" and "acid soluble" phosphate, particularly in blood sera with elevated phosphorus. The Autoanalyzer

results always are lower. Extensive studies indicate that calcium and protein complexing probably prevent some of the phosphorus from dialyzing. We have been unable to clarify the significance of this observation.

The addition of a moving chart recorder to the Coleman Flame Photometer and the Beckman B. Spectrophotometer to facilitate the recording of data is being studied. Flow-through type cuvettes have been developed for several spectrophotometers which will increase the speed and efficiency of measurement and recording. The serum magnesium determination has been revised, and more satisfactory experimental results are now being obtained with thiazol yellow techniques.

The dye hydroxybenzeneazobenzoic acid, used for the determination of albumen by a procedure originated in this laboratory, is again being studied, because in rare cases the dye does not bind albumen quantitatively. The cause of this interference is not known.

The Control Chemistry Service is conducting a long-term study of chemical and electrophoretic variations in the serum protein of normal individuals; comparing the findings under conditions of bed rest with those obtained during normal activity. This is necessary because the diagnosis and treatment of certain diseases depends partly on comparison of protein components and distribution in patient's serum with that in normal control sera. Finally, a study of the various factors influencing electrophoretic mobility and dye absorption of proteins in paper electrophoresis is being made. This technique is becoming established as a routine laboratory procedure, and there is still need for critical evaluation of the experimental factors involved.

The Microbiology Service has added several new media for the cultivation and differentiation of micro-organisms. A synthetic medium is being evaluated for the cultivation of tubercle bacilli from pleural fluid, and other media are being tested for the differentiation of saprophytic acid-fast bacilli from the tubercle b acillus. A single medium for the isolation and differentiation of coagulase positive, mannitol positive, and pigmented Staphylococci is being developed. The technique of slide culture of fungi is being incorporated as a routine procedure, and a permanent library of fungus slides has been started.

A new instrument for multiple dispensing of Staphylococcus phages was developed by Mr. Zierdt, Dr. Fox, Dr. Peters, and the staff in the Instrument Section. Mr. Zierdt evaluated this device and demonstrated that it greatly diminishes the time required for routine phage typing. Complement fixation tests for mycotic infections, and fluorescent antibody techniques are being developed for eventual addition to the armamentarium of routine diagnostic procedures.

One of the most important contributions of the Microbiology Service has been the compilation of a laboratory manual detailing all the methods of analysis for bacterial cultures used in our laboratory. This effort, which is beyond the usual duties of our staff, was accomplished by the hard work of several of our technologists with the aid of our residents.

Awards

The uniform cooperation among our entire staff has enabled this department to perform its functions in spite of heavy overloading. Certain individuals and groups were nominated for special recognition for their unusual ability to assume responsibility, manifest individual effort and initiative, and demonstrate superior performance.

A group in Microbiology was commended for its continuing enthusiastic devotion to the extremely high standards required for the performance of duties in this area. In addition, members of the group have excelled in the training of new personnel, worked with clinicians in evaluation of tests and the development of diagnostic mycology.

A unit of the Hematology Service has done a superlative job on the routine and developmental hematological tests. They have demonstrated a high degree of informed interest in the scientific objectives of the department. In addition, they have kept informed by reading current literature, have conducted selected groups of newly developed tests; have assisted in evaluation of new tests from other research organizations, and consistently accomplished research in the development and utilization of new procedures and tests.

In Clinical Chemistry, six staff members have received recognition for their superior performance. In particular, their application of a thorough knowledge of analytical organic and inorganic chemistry to the practical problems of the laboratory, their continuous evaluation of precision and accuracy of methods used, and the development of improved methods as aids to diagnosis and treatment have contributed immeasurable benefits to patient care.

In addition to the group awards, the following individuals received separate awards for contributions of special acts and services to improved scientific methods and their application.

Mr. Charles Zierdt has improved a technique for production and preservation of typing phage by several modifications and innovations of the cultural propagation, lyophilization, preservation, storage and reconstitution of bacterial phage used in typing strains of staphylococcus. This work was originated voluntarily by him and was performed after hours, as well as during the regular work day.

Mrs. Vivian Arnold, secretary to the Chief of the Department, in addition to carrying out efficiently her own assignments, has displayed an exceptional background of knowledge, an unusual degree of tact and diplomacy, and has acted as a central check point for almost every phase of the varied fields served in the entire department.

Miss Anne Jennings was the first technologist to be employed in the Clinical Pathology Department at NIH. She was, indeed, the only one who was available before the opening of the Clinical Center Building to assist the Chief and his meager professional staff in the arduous task of ordering equipment, and making ready the first diagnostic laboratories

when the building was opened to patients on July 6, 1953. Her continued interest and concern as chief technologist in Microbiology has furthered the functions of this department.

Mr. Hiroshi Nishi has continuously demonstrated his originality and ingenuity in the development and application of new methods in Clinical Chemistry. In addition, he has modified many standard methods for application to this laboratory.

1958-1959

	July	Aug.	Sept	Oct.	Nov.	Dec.	Jan.	Feb.	March	Apr.	May	June	Ave.	Total
Av.Pt.Cons.	386	393	383	409	390	349	389	404	406.7	403.0	396.8	406.7	393	4716
Pt.Workdays	8492	8253	8043	9407	7020	7329	8169	7676	8947	8866	8833	8947	8292	99482
Test Proced.	28432	28277	27430	31991	24420	25048	30409	28804	31192	32587	26882	30834	28858	346306
Test/Pt/Wkdy	3.34	3.3	3.3	3.3	3.4	3.3	3.6	3.6	3.4	3.5	3.2	3.4	3.4	
Test/Pt.	73.5	70.1	69.7	76.0	60.6	69.5	76.3	68.8	74.3	77.8	66.3	73.6	71.3	856.5
Tech.Staff	49	50	45	46	47	45	32.7	39.8	36.4	37.2	37.4	43	42.3	
Test/Tech/Dy	26.3	26.9	29.0	30.2	28.8	26.4	44.3	38.1	39.8	39.8	34.2	33.9	33.1	

Average Tests/Patient

	July	Aug.	Sept	Oct.	Nov.	Dec.	Jan.	Feb.	March	Apr.	May	June	Ave.	Total
NCI	120	112	107	133	98	109	110.2	101.8	108.2	142.3	104.9	111.7	113.2	1358
NIAID	117	117	109	114	105	134.8	124.8	137.8	132.4	125.7	135.7	114.3	122.4	1468
NHI	66	71	64	64	70	73.8	79.7	61.5	72.9	67.3	65.3	84.7	70.1	841
NIAMD	66	66	71	73	51	73.8	89.9	68.6	65.6	66.0	55.4	60.3	68.1	806
NINDB	32	33	42	40	32	34.8	32.9	36.7	45.6	35.1	33.1	33.1	36.9	431
NIMH	3	7	5	2	2	3.0	1.9	1.9	3.6	3.1	1.1	8.9	3.5	43.0
NIDR	128	82	124	85	62	83.7	82.3	111.7	124.9	67.1	80.8	193.2	103.9	1247

	JANUARY-JUNE 1958		JANUARY-JUNE 1959			
	TOTAL (6 mos)	AVERAGE/MONTH	TOTAL (6 mos)	AVERAGE/MONTH	TOTAL CHANGE 6 Mos/Period	% INCREASE
Ave.Pt.Census	2,355	392.6	2406.1	401.1	+ 51	+ 1.9%
Pt.Workdays	49,598	8,266	51,438	8,573	+ 1,840	+ 3.8%
Test Proc'ed.	151,311	25,519	180,708	30,118	+ 29,397	+ 19%
Tests/Pt/Wkdy		3.0		3.4		+ 13.3%
Test/Pt.	387.5	64.6	437.1	72.9	+ 49.5	+ 12.8%
Tech Staff		38.1		37.7		+ 1%
Test/Tech/Day		31.4		39.3		+ 25%
NCI	622	103.7	680	113	+ 58	+ 9.3%
NIAID	571	95.1	770	128	+ 199	+ 35%
NHI	329	54.9	432	72	+ 103	+ 31%
NIAMD	379	63.1	406	69	+ 27	+ 7.1%
NINDB	185	31	216	36	+ 31	+ 9.7%
NIMH	37	6.1	21	3.6	+ 16	+ 43%
NIDR	521	87	682	114	+ 161	+ 30%

PERIOD FROM JULY TO OCTOBER 1959
AS COMPARED WITH THE SAME PERIOD 1958

	July-October 1958		July-October 1959		Total Change 6 mos/Period	% Change
	Total(4 mos)	Average(/mos)	Total(4 mos)	Average(/mos)		
Average Pt. Census	1,571	393	1,527	382	- 44	- 2.8%
Pt. Workdays	34,195	8,549	33,187	8,297	- 1,008	- 2.8%
Test Procedures	116,130	29,085	116,480	29,120	+ 348	+ 0.3%
Tests/Pt/Wkdy		3.3		3.5		+ 6.0%
Test/Pt	289	72.3	298	74.5	+ 19	+ 3.0%
Tech. Staff		38.0		43.1		+ 13%
Test/Tech/Day		35.0		31.8		- 8.5%
			AVERAGE TESTS/PATIENT/PERIOD			
NCI	472	118	441	110	- 31	- 7.0%
NIAID	457	114	503	126	+ 46	+ 10%
NHI	265	66	327	82	+ 62	+ 24%
NIAMD	276	69	280	70	+ 4	+ 1.4%
NINDB	147	37	136	34	- 11	- 8%
NIMH	17	4.2	38	9.5	+ 21	+ 126%
NIDR	419	105	338	84.5	- 81	- 20%

attachment 1
Serial No. CC-

Research Projects

Clinical Pathology Department

1959

CP 59-01 Cell Volume and Size Distribution Patterns in Ascites Tumor and Tissue Culture Populations

A modified Coulter Electronic Counter was utilized to count and size larger cells, such as mouse ascites tumor cells and cells growing in tissue culture as described last year. The cells displace their volume of electrolyte, thereby producing proportionate changes in resistance of an electrolyte column in the aperture of a tube between electrodes. Adjustment of the circuit characteristics permit enumeration of only those cells larger than a selected electrical threshold, which is proportionate to a size limit of the cells. A series of counts at different adjustments permits the construction of a size frequency distribution curve. 5,000 cells may be measured and counted in a few minutes. This project was completed during 1959, and a manuscript has been submitted for publication in the Journal of the National Cancer Institute. Tissue culture cells are readily counted and sized, particularly those grown in free suspension. Hela culture cells possess a median volume of 2800 μ^3. Several ascites tumor cells of mice were sized and found to range from 500 μ^3 to 1800 μ^3 for different types of cells. This instrumentation and method of counting large numbers of cells quickly and of determining cell volume distribution patterns is useful in examining tissue cell cultures, bone marrow suspensions from patients, peripheral blood suspension for size distribution and counting of white cells in leukemias and red cells in various types of anemia, and research on animal tumor cells.

G. Z. Williams
A. C. Peacock (NCI)
H. F. Mengoli

CP 59-02 Cytochemical Studies of Human Marrow by Ultraviolet Television Microscopy

The studies of ultraviolet absorption morphology related to the chemical composition of various parts of the cells, such as proteins, nucleoproteins, etc., in normal and abnormal bone marrow, has been continued during the current year. This information is being accumulated for an atlas of the ultraviolet microscopic patterns of cell types and distinctive changes in different abnormalities involving the marrow.

G. Z. Williams
G. Brecher
F. A. Fox

CP 59-03 Properties of Acridine-Orange as a Supravital Stain for Bone Marrow and Tumor Cell Suspensions

The studies of the application of acridine-orange in fluorescence microscopy for differentiation and identification of distinctive morphological patterns in tumor cells and human marrow cells have been continued. We have also continued to compare the fluorescence and the ultraviolet absorption patterns in different types of cells.

G. Z. Williams
A. C. Peacock (NCI)

CP 59-04 Cytochemical Determination of Phosphatases in Living Cells

When formazan phosphate taken up into the cytoplasm of normal living cells is observed under the ultraviolet microscope, it appears that the phosphate is split off, thereby leaving insoluble formazan which precipitates in situ. This is observed as it occurs in cells, and time-lapse motion pictures can be made of the process. The cause of the splitting off of phosphate is under study to determine whether or not this is due to activity of a phosphatase. The formazan phosphate was prepared by Dr. Peacock.

G. Z. Williams
A. C. Peacock (NCI)

CP 59-05 Biological Reactivity of Substituted 2, 3, 5 Triphenyl Tetrazoliums

The work on determining the properties of the tetrazoliums in relation to biological activ ity causing their reduction to formazan, has been continued during the current year. Further evidence has been obtained for a difference in the reduction characteristics of several groups of substituted tetrazoliums. Several more derivatives of tetrazolium were synthesized by Dr. Pierce to attack side chain groups in different positions, to fill in the series of these compounds and clarify the influence of the nitro and methoxy groups in the reactivity of these compounds. The measurement and depiction of the reaction rates of several of these compounds in single living cells under the ultraviolet microscope have been accomplished by time-lapse motion picture photography, and sequential measurements of absorption patterns of the same cells with the oscilloscope. It was found that the several classes of tetrazoliums react at different rates in fresh liver cells. These studies are being continued. A report of this work on reaction rates in living cells was made at the Ciba Cytochemistry Symposium in Copenhagen, Denmark, on April 2 and 3 of this year.

G. Z. Williams
A. C. Peacock (NCI)

CP 59-06 Improvements in the Ultraviolet Television Microscope for Time-Lapse Cinematography

Several improvements for accomplishing critical and properly synchronized time-lapse cinematography were completed during the current year, so that the instrument now operates satisfactorily and satisfactory time-lapse motion pictures have been made of tissue culture cell changes, in response to ultraviolet light injury and the progression of reduction of tetrazolium to formazan within the cytoplasm of individual cells. This system was reported at the Twelfth Annual Conference on Electronic Techniques in Medicine and Biology, Philadelphia, during November of this year.

G. Z. Williams
R. Neuhauser (RCA)
G. Johnston (Instrument Section)
J. Vurek (Instrument Section)

Cooperative Research with other Institutes and Individuals

The following numbers designate the cooperative research projects detailed above with the Office of the Assistant Director of Research, NCI and the Field Investigations Branch, NCI.

CP 59-01
CP 59-03
CP 59-04
CP 59-05

CP 59-07 A Study of the Factors which Influence the Binding of the Dye Hydroxybenezeneazobenzoic Acid with Protein, with Particular Reference to Human Serum Albumin

This is the dye used for the determination of serum albumin by a procedure developed in this laboratory. Rarely is a serum encountered in which the albumin does not quantitatively bind the dye. It seems that the albumin of these subjects is either qualitatively different from normal albumin, or that some of the dye-binding sites are occupied by other molecules which cannot be displaced by the dye. The exact cause of this interference has not been ascertained, and the investigation is being continued.

A. T. Ness
H. Dickerson

CP 59-08 The Measurement of the Concentration and Relative Distribution of Human Serum Proteins by Electrophoretic and Chemical Methods on Normal Subjects in Active and Resting States

Diagnosis and treatment of certain diseases are partly based on serum protein analyses of patients, compared to protein analyses

on normal active subjects. Because most patients are bed resting, it is necessary to determine values on normal subjects who are also in the resting state, if comparative data is to be properly assessed. It has been observed that out-patients show a significant difference in relative distribution and concentration of serum proteins compared to in-patients, and seemingly independent of the disease state.

This study is being continued for several years. Very few normal persons are put to bed for several days, and the accumulation of this data will be slow.

A. T. Ness
N. Dickerson

CP 59-09 A Study of the Factors Which Affect the Electrophoretic Mobility and Dye Absorption of Proteins in Paper Electrophoresis

Paper electrophoresis is rapidly establishing itself as a routine procedure for determining the distribution of serum proteins. It is important to evaluate critically the experimental factors involved in this technique, since the voluminous literature of recent years has reported confusing and conflicting results.

A. T. Ness
N. Dickerson

CP 59-10 *In vitro* studies were tritiated thymidine on synthesis of DNA by Leukemic and Benign Cells
(Sponsored by NIAMD and NCI in cooperation with Dr. L.R.Schroeder,NCI)

The previously described *in vitro* technique identifies cells in the stage of DNA synthesis at the time of their removal from the body. Experiments during the past year strongly suggest that under the conditions used there is neither actual multiplication of cells nor entry of new cells into DNA synthesis, so that the percentage of labeled cells accurately reflects the proliferative potential of the various cell types present in the marrow or peripheral blood at the time the samples are removed from the body. It is hoped that true correlation can be established between this proliferative potential of leukemic cells in individual patients and the response to treatment. Very preliminary results suggest that patients with initially few cells in active DNA synthesis may respond less favorably than patients with a high proliferative potential. Parallel experiments on patients with infectious mononucleosis have indicated that these benign cell lines have a proliferative potential comparable to that of acute leukemias, and it is concluded that the degree of proliferative potential *per se* is not characteristic of malignant cells in the hemopoetic system.

Plans for the coming year consist in confirming these observations on an adequate number of patients, and to explore the suggested negative correlation between proliferative potential of leukemic cells and the response to chemotherapy.

G. Brecher

CP 59-11 Abnormalities of the Blood Coagulation Mechanisms of Patients undergoing Perfusion With the Mechanical Pump-oxygenator During Cardiac Surgery

It has been previously shown in this laboratory that part of the post-operative bleeding tendency of patients involving surgery with extracorporeal circulation can be prevented or corrected by careful tritiation of the protamine dosage, so that all of the injected heparin remains fully neutralized. There still occur, however, occasional unexplained postoperative bleeding difficulties. Patients undergoing this type of extracorporeal circulation are now being studied with the aid of the thrombelastograph in the hope of discovering the nature of their suspected, but hitherto unidentified, coagulation defect.

J. R. Little

CP 59-12 An improved model of the Coulter Counter has been purchased which permits rapid sizing of cells. Experiments are still in the very early stages, but it has already been possible to construct platelet size distribution. This type of work is essential to any subsequent counting of platelets by electronic means of the Coulter Counter type, where discrimination of particles to be counted from background or contamination is a matter of knowledge of size distribution. It is not yet possible to assess the potential significance of this research, but it will at any rate establish whether the Price-Jones curves of RBC, WBC and platelets have any diagnostic value. Hitherto, such curves have been available only for RBC, and even there only to an insignificant extent because of the laboriousness of prior methods for construction of such distribution curves.

G. Brecher

CP 59-13 Thrombocytopenia in Guinea Pigs with Delayed Hypersensitivity Reactions. (In cooperation with Dr. Sanford Stone and Jules Freund, NIAID)

Guinea pigs immunized against albumen with the aid of Fruend's adjuvant, and later challenged by large doses of albumen, exhibit a mixture of immediate and delayed hypersensitivity reactions, some into extensive hemorrhagic phenomena. Prolonged thrombocytopenia was found in the pigs with hemorrhagic phenomena, but quite comparable drops were observed in animals without hemorrhagic skin reactions. The correlation between thrombocytopenia and these hemorrhagic reactions, whether casual, predisposing or merely coincidental, is being explored.

G. Brecher

Calendar Year 1959

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

REHABILITATION DEPARTMENT

CC-38
Serial No.

During calendar year 1959, the Rehabilitation Department continued to carry out its primary function: The treatment by use of recognized measures of physical, occupational and speech therapy of patients referred by Institute physicians. It has provided Institute physicians with measurements and tests for determining diagnosis, neuromuscular status, functional ability and progress; and for evaluation of drug or other therapy.

As previously reported, the Department's Physical Therapy Service provides the following services:

1. Tests and measurements:
   a. Manual muscle evaluation.
   b. Joint range of motion measurements.
   c. Girth, length and expansion measurements (linear, volumetric).
   d. Electrodiagnostic test with constant current impulse stimulator to determine chronaxia and strength-duration curve.
   e. Faradic-galvanic test for nerve or muscle response.
   f. Self-care evaluation (Activities of Daily Living).
   g. Skin temperature and oscillometric tests.
   h. Vital capacity determination with Collins Spirometer or portable McKesson.
2. Heat, in various forms including:
   a. Superficial - hot packs (hydrocollator), infrared, paraffin bath, whirlpool.
   b. Deep - short wave diathermy, microwave, ultra-sound.
   c. General body heat - Hubbard tank, Moistaire cabinet.
3. Therapeutic exercises:
   a. General exercise - passive, active assistive, active and resistive.
   b. Muscle re-education, using Warm Springs techniques, mass movement patterns and proprioceptive facilitation techniques.

- c. Ambulation training, including elevation, with and without parallel bars, braces, crutches, canes.
- d. Posture exercises.
- e. Underwater exercises (Hubbard tank, therapeutic pool).
- f. Progressive relaxation.
- g. Pre- and postoperative thoracic surgery program of breathing and coughing and range of motion exercises.
- h. Pre-prosthetic and prosthetic training.
- i. Training in self-care activities.
- j. Exercise with apparatus such as weights and pulleys, shoulder wheel, finger ladder, quadriceps boot, stationary bicycle, restorator, and Elgin exercise table.
- k. Breathing exercises and postural drainage instruction.

4. Miscellaneous:
    - a. General and local application of ultra-violet light.
    - b. Contrast baths.
    - c. Scientific massage.
    - d. Neck traction (Sayre).
    - e. Compression bandaging.
    - f. Application of splints and casts to maintain joint in good anatomical and functional position.
    - g. Application of splints and casts to correct joint deformity.
    - h. Instruction to patients and family in home care program (application of heat, exercise, use of self-care aids).
    - i. Fitting and dispensing canes and crutches.
    - j. Prescription and procurement of corrective shoes, braces, corsets, splints, prostheses.

Services in Occupational Therapy include:

1. Maintaining or increasing joint range of motion and muscle strength; improvising coordination; and developing endurance and work tolerance, through utilization of appropriate technics.
2. Testing and training patients to aid them to become self-sufficient in eating, dressing, homemaking, and the other significant activities of daily living.

3. Exploring patient's skills, aptitudes and work habits in order to provide basic data for vocational planning.
4. Observing and recording patient behavior patterns for use as diagnostic aids or in evaluating the patient's reactions in specific research studies.
5. Substituting constructive interests for the insecurity and anxiety which may develop during the research study.
6. Providing normal developmental learning experiences for children.

The Speech Therapy Service provides:

1. Speech language and audiometric evaluation.
2. Stimulation and education or re-education of language and language concepts.
3. Exercises for improved control and coordination of the speech musculature.
4. Breathing exercises.
5. Auditory training.
6. Articulation therapy.
7. Voice therapy.
8. Teaching of post-laryngectomy speech.
9. Observing and recording patient behavior patterns, for use as diagnostic aids in specific research studies.
10. Counselling patients' families in regard to speech problems and home procedures following discharge, and providing information regarding availability of speech therapy services in the home vicinity.

The program of application of plaster casts for patients having rheumatoid arthritis, and for other patients, has continued to increase in volume and in value. During 1959, it is estimated over 200 casts or splints will have been applied. We have considered means of providing corrective or supportive splints which have longer life than that afforded by plaster, effective as the latter has been. Toward this end we have had demonstrations, and have procured a supply of Dura-Foam plastic which is very durable, light in weight, can be cleaned and is more acceptable cosmetically than plaster.

REHABILITATION DEPARTMENT

A minimal amount of new equipment was acquired during the year. We purchased the Circ-O-Lectric Bed which the Physical Therapy Service had on a trial basis for some months. This bed functions as a tilt table, with many variations of reclining, sitting, and vertical postures; and includes the advantages of the Stryker frame, enabling the patient to be turned from prone to supine position and vice versa.

Equipment requisitions which we expect to submit in the future will cover:

1. An electromyograph - a tool of increasing importance in the adequate functioning of the departments of physical medicine and rehabilitation in general hospitals and of rehabilitation centers.
2. Models of the upper and lower extremities which demonstrate muscles, nerves, blood vessels, bones and joints. Such models are most effective for in-service training and for ready reference.
3. A tape recorder and an audiometer for use in speech therapy.

During 1959, the Department was engaged in the production of a handbook for arthritis patients. This cooperative project has had as other participants the Arthritis and Rheumatism Foundation, the Division of Special Health Services and the National Institute of Arthritis and Metabolic Diseases.

It has been our privilege during the past year to meet and confer with a number of physicians from abroad, who are charged with the administration of important rehabilitation programs in their respective countries. Some were referred by the State Department or by the Office of Vocational Rehabilitation. In selected cases we have provided suggested layout plans and equipment lists for physical therapy departments.

The problem of space appears to be an ever-increasing one. This is not because of our expanding requirements, but because of requirements in connection with the new surgical wing. It appears that cordidors to such a wing will take one module from Occupational Therapy (4th floor, D wing), and comparable space from Physical Therapy on the

fifth floor. Three modules were previously lost to Occupational Therapy when they were turned over to Clinical Pathology in 1958. The loss of another module will necessitate certain partitioning. In the Physical Therapy area it appears that space is to be taken solely for access to the roof of the new surgical wing. We have previously suggested the possibility of using our space as it is presently constituted for ingress and egress. If, for any reason, this is not feasible, considerable reconstruction will become necessary in Physical Therapy since the space involved is not a module but of permanent construction. Planning and budgeting should be considered for the many changes which will be involved.

A significant strengthening of the Occupational Therapy program resulted early in the year, when a therapist was employed to spend full time in the Arts and Crafts Clinic. This Clinic previously had been run by volunteer Gray Ladies who, although rendering valuable service, lacked the skills of professional therapists, and whose attendance was often sporadic. Later in the year another therapist was employed to take over the program for bed patients, which previously had been covered by the Chief of the Service and a staff member in addition to their other responsibilities.

Other personnel changes have occurred in both Physical and Occupational Therapy Services, with some resignations and replacements. At the close of the calendar year, each of the Services finds itself with two vacancies.

During the calendar year 1959, Mr. Lamont-Smith, staff physical therapist, attended a two-weeks' course (Neurophysiological Basis for Rehabilitation) at the University of Pennsylvania. We have previously expressed the belief that such training should be provided for selected staff members, and hope that a reasonable program can be formulated to enable us to take advantage of the newer knowledge and technics of rehabilitation medicine.

We would like to recommend again the provision of a small number of beds for Clinical Center patients ready for discharge by the Institutes, by reason of the

completion of the research study, but who are still in need of rehabilitation services.

Annexed hereto are statistical reports for Physical Therapy Service, Occupational Therapy Service, and Speech Therapy Service.

Calendar Year 1959

Serial No. CC-42

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

PHARMACY DEPARTMENT

The Pharmacy Department comprises: the Office of the Chief; Pharmacy Service; Central Sterile Supply Service; Pharmaceutical Development Service; Radiopharmaceutical Service. Activities have been expanded during the year to provide further services to the clinical area with every effort being made to maintain high standards.

PHARMACY SERVICE

The Pharmacy Service has continued to improve pharmaceutical service wherever possible. Packaging research has been continued in the department and in this service, in an effort to provide more efficient packaging with less personnel time. The basic idea in the continuing search is the need for a more usable and suitable package by the clinical area.

A new narcotic ampul container was developed and placed into use during this year, which greatly improved the storage of narcotic ampuls on the nursing units. It also aided in accounting for the narcotics at the change of each eight hour shift. A photograph of this is included in the report.

A new style glass bottle was placed in use after considerable packaging research for the dispensing of tablets and capsules. This contains a snap-on polyethylene cap which is covered by means of an aluminum seal, therefore making the item tamper proof. The polyethylene snap-on cap without the aluminum seal is used for the majority of the outpatient activities where the size of the medication permits. This is a safer cap, than the plastic screw cap, as far as children's medications are concerned, in private homes.

The Pharmacy Service dispenses the majority of the investigational use drugs to the various nursing units. The Pharmaceutical Development Service is responsible for receiving and registering drugs,

and for establishing proper labeling techniques and procedures. Drugs are then dispensed on individual prescription from the Pharmacy Service. This eliminates dispensing of routinely available investigational use drugs, and the keeping of their records from the developmental area. The dispensing of investigational use drugs is not considered to be routine in the majority of pharmacy services in other hospitals. However, our pharmacy service staff is capable of performing this very satisfactorily, and, this does require an above-average staff. At the present time there are 258 different lots of investigational use drugs dispensed by the Pharmacy Service. They have, of course, been received, registered and label format established by the Pharmaceutical Development Service. This represents 161 separate compounds of 214 different sizes and forms of drugs. There are, at present, nine separate double blind studies being supplied from the prescription area in the Pharmacy Service, both on inpatient and outpatient basis.

Pharmacy Service personnel prepare intravenous solutions by adding additives in an extemparaneous fashion. These include products which are more efficiently cared for in the Pharmacy Department, and are properly labeled with the amount of material added and a deadline for use, in order to prevent sterility and protect the safety of the patient. Two examples are: Penicillin and Heparin are added to an intravenous solution for the Allergy and Infectious Units; Antibiotic irrigating solutions are being prepared in the Pharmacy on an extemperaneous individual prescription basis.

All ophthalmic solutions are being supplied sterile from the Department. Sterile ophthalmic solutions were being supplied from the Pharmacy Department for inpatient use in the 1958 report. In 1959, the Department has extended this to extemperaneous prepared solutions for both inpatient and outpatient use.

In order to improve medication safety, the 30 ml. vials of Codeine were replaced with two strengths of 1 ml. Codeine Phosphate ampuls. This reduces the possibility of bacterially contaminated solutions being administered to patients, in addition to simplifying the record keeping.

Plastic squeeze spray type bottles are being supplied for several types of medications and are much superior to glass containers, from which the medication had to be transferred to an atomizer. This allows the labeled container to be used as a spray. Another packaging research project brought to final conclusion was the use of tote boxes with polyethylene plastic liners for delivering supplies. It eliminates the washing of metal baskets between uses. It further eliminates the possibility of cross contamination by having used, or contaminated, materials returned in the basket which is resent to another nursing unit. These liners are used for one round trip, and are disposed of and replaced with a new liner for each use.

Forms have been continuously revised during the year. The most notable is the new prescription blank which has added several new features. It allows the Pharmacy Department to use generic name dispensing more effectively with a check block for the exact trade marked item, where it is necessary. It further allows for the name of the medication to be placed on the container for an outpatient prescription label, if the physician so requests. It has allowed the physician his individual discretion, yet it has made it considerably easier to obtain this information if he cares to have it added. It provides space for the number of days of medication the physician requests, allowing the Pharmacy to dispense the exact amount--eliminating the overage or shortage which frequently occurs on an outpatient prescription.

A procedural manual for the handling of pharmaceutical service items has been completed, and is available to each service chief in the Department.

The Pharmacy Service total workload increased approximately 7% during the fiscal year. However, the prescription increase amounted to approximately 36%. To a great degree this was caused by the issuing of investigational drugs through the Pharmacy on a prescription basis. The great increase in prescriptions did not upset the staffing pattern or service available from the Pharmacy, due to the fact that the dispensing area was completely redesigned. By redesigning the prescription area and relocating equipment, it was possible to prepare and issue the increased number of prescriptions with less professional time. Only with this more efficient move has the Pharmacy been able to keep up with its demand for prescription service. Any further increase or emphasis on individual compounded prescriptions will necessitate the assigning of an additional pharmacist to this area. This redesign and relocation was based upon observations made during a visiting tour of other hospitals.

A safe file was procured for the storing of prepackaged narcotics for the nursing unit areas. This has reduced floor space used and has, therefore, allowed us to relocate this activity to make it more efficient. The nursing unit dispensing area has been completely rearranged, but a few cabinets which have been ordered to complete this rearrangement have not arrived. The Service has broken from the traditional way of storing prepackaged medications, such as alphabetically; and has moved all types of fast moving items into one area. The majority of the nursing unit requisitions can be filled from a small portion of the area. The remaining 25% or 30% are filled from the remainder of the cabinets.

Very necessary for the Pharmacy Service and for the Department is additional space for a combined and improved packaging service. The Pharmacy Service has been relying upon using so-called "left over" space, in order to package tablets, capsules, certain liquids and oint-

ments. It would be more efficient to have an area designed for packaging for the Pharmacy Service; for a portion of the Pharmaceutical Development Service, and for certain types of packaging for the Central Sterile Supply Service. It is hoped that more space can be obtained for this activity in the very near future in order to increase the efficiency of the Department. Pharmacy Service also has a great need for an ultra-violet hood so it may prepare more of the extemporaneous prescriptions for intravenous use and for ophthalmic preparations. The use of the one existing ultraviolet hood by two services has many difficulties, and has resulted in reduced service to patients due to a clashing of schedules. This ultraviolet hood should be located in the additional space for a specialized packaging service and become a portion of this activity.

PHARMACEUTICAL DEVELOPMENT SERVICE

While the number of requests for the Pharmaceutical Development Service have been somewhat reduced from the last fiscal year, the overall number of units have increased. The requests have been lessened to some extent due to the fact mentioned in the 1958 report, where requests for short services have been referred to the Pharmacy Service. The Pharmaceutical Development Service has limited its activities to projects such as injectables, or those which require in excess of four to six hours of minimum preparation and development time. Several developmental projects have taken many days and weeks. The activities carried on by the Pharmaceutical Development Service include testing Central Sterile Supply items and solutions for sterility. Chemical analysis of some of the preparations of the Department have been carried on.

They enter into the preparation of randomization schedules with principal investigators of several institutes, involving single and double blind studies. The Service has carried on research and development in the chemical-physical property study of such items as p-anisaldehyde thiosemi-carbazone, Diphenyl Thiocarbazone, Androstane-17B-01 Acetate. Such information was not immedietely available or was unavailable in the literature.

The Service carried on a project of solubilizing basic materials where the information was not available in the literature or available from the primary source. Examples of these are 4-amino antipyrine, 6-Azauradine, Methotrexate, Dicholoromethotrexate, Liothyrione and Acinomycin-D.

In cooperation with the Endocrinology Branch of the National Cancer Institute and the Division of Biologic Standards, they conducted a preliminary study evaluating the suitability of ethylene oxide sterilization of growth hormones. This has great potential and will be pursued further.

They have continued to cooperate with investigators, and in some cases have produced pilot batches of products to be used for animal experimentation, where it has definitely been shown that the next step in the use of the product will be in humans. The animal pilot production has been used as a method of arriving at proper formulations prior to clinical trials.

In cooperation with the Dermatology Service of the National Cancer Institute, a project was instituted for extraction of a staining principal from the green hulls of black walnuts. This has presented a problem in maintaining a supply of green hulls of black walnuts, so as to provide a year round supply of the staining preparation.

Suitable space and equipment is badly needed for certain activities in preparation of products by the Pharmaceutical Development Service. A separate room or cubicle is becoming more necessary for preparing potent pharmaceutical products which have not had their toxicity established. It is felt that there is a great need for this, and that service has been hindered in several areas. Also, the Department is unable to certify that some of our personnel have not, or could not be, injured by these products. This eventual outcome of a slow buildup of toxic materials may not become evident for some years. An isolated room under negative pressure is sorely needed for this activity before personal injuries result.

CENTRAL STERILE SUPPLY SERVICE

The Central Sterile Supply Service total issues increased by approximately 8% during the last calendar year. There has been no increase in personnel to offset this increase. The Service is continuing to evaluate new types of packaging techniques; continues to do research in newer packaging materials, and into more improved disposable materials when they become available. Our primary concern is that the product must be economically suitable for the end user, be packaged so that it is completely usable, and so that sterility can be guaranteed to the end user. When this is not possible, the product is not used and the requisitioners, who may desire these types of items, are told why. It is felt that the evaluation of materials in this fashion, and dissemination of this information, is a very important service to the end user.

The problem of working with more complicated special items continues to be an ever increasing activity in the Service. The special handling materials are increasing and becoming more complicated, to the point where non-professional personnel, such as are utilized, cannot process all of the materials. Therefore, the clinicians or other professional personnel caring to use these items, do the actual processing and packaging. The Service offers a central

sterilizing service for this type of material.

Recently, a small model ethylene oxide gas sterilizer was transferred from the Sanitary Engineering Branch. This is being utilized for certain materials which are heat labile and certain other products which cannot be autoclaved. It replaces some of the "cold sterilized" processes. However, we cannot process all materials which should be sterilized in this fashion. The need exists for a larger model combination gas and steam sterilizer, as has been mentioned in earlier reports. This larger model would have advantages in making more suitable material available for use, and in lengthening the life of many of the supplies. It would make available many new products which the Department has not been able to offer, due to the fact that they cannot be properly packaged and sterilized.

A semi-automatic packaging machine is even more desperately needed as more special items for processing are requested, requiring personnel to be shifted from the routine processing of supplies. It has been demonstrated by pilot runs, on a commercial machine, that this type of packaging is very suitable and desirable for the Clinical Center. The pilot run was made upon the identical machine which is requested for the Clinical Center Central Sterile Supply Service of the Pharmacy Department.

The main hinderance has been space in which to place such a packaging machine. There is a great need for the Blood Bank area, when this area is vacated, to house a central packaging service which would include dressing packaging by the semi-automatic packaging machine; central drug packaging of the Pharmacy Service, and a portion of the Pharmaceutical Development Service packaging. In addition, it would allow an area for the processing of materials of unknown toxicity and an area for an ultra-violet hood.

One of the biggest problems has been the extra workload caused by the changing of techniques in the surgery suite. The workload for this particular section of the Central Sterile Supply Service has increased over 100% from the corresponding period for the past year. This had to be adjusted by internal shifts of personnel from other activities, which have had to be reduced to some extent in order to compensate. It has necessitated the use of overtime rather frequently, in order to accomplish this increase in workload for this particular activity.

RADIOPHARMACEUTICAL SERVICE

The Radiopharmaceutical Service activity has increased considerably since the last fiscal year. The total number of requests have not risen abruptly, although the total service and complexity of the

service offered has increased amazingly. For the most part, services rendered by the Service are not available from industrial sources. For this reason, the monetary value of products compared to those from other sources could not be assessed properly. However, on the formulations which are commercially available, and from which a formulation has been prepared from the basic material, a rather sizeable saving has been effected. In addition to offering services which had not previously been available in pharmaceutical quality, it is felt that the formulations made from basic products have afforded savings much in excess of the salaries involved. In addition, products not available from any other source have been prepared which cannot be properly assessed but undoubtedly would add to these savings. The clinicians have consulted with the Pharmacy Department concerning radiopharmaceutical procedures on an ever increasing scope.

In certain cases, considerable research was necessary on the part of the Services to produce the product which conformed to the needs of the clinician. One notable instance was a radioiodinated Gamma Globulin with a high macro globulin content. The clinicians had been unable to produce a satisfactory product in their laboratory; industry had been unable to produce a satisfactory product; but after several conferences and some months of research, the Radiopharmaceutical Service furnished to the clinician a satisfactory product which retained the high macro globulin content during formulation and processing. A Hydrocortisone-4-C-14 was prepared in a suitable pharmaceutical preparation for a clinician. This product cannot be procured from any other source. When this type of service is available to clinicians, there is every indication that there will be more and more demands for service upon this portion of the Department. It is predicted that there will be an ever increasing demand which cannot be met with the present staff for this Service. Therefore, service must be limited to a certain number of projects which can be carried on at one time, and others have had to be delayed until they may be scheduled. On July 1, 1959, the Clinical Center Director's memorandum, titled "Control of Radioactive Drugs and Materials For Human Use" was issued. It is felt that the full impact of this has not been felt to date. The Pharmacy Department has not policed this controlling mechanism, nor is it felt that such a policing function should be ours. However, it might be beneficial if the Radiation Safety Office, the Clinical Center Administration and the Pharmacy confer to establish means of administering the full intent of this memorandum. However, there is a feeling that there is such a reservoir of activity which has not yet been checked, that the Pharmacy Department, through its Radiopharmaceutical Service, could not possibly handle the patient administered radioactive compounds presently being used. It may be wise to police and enforce the intent of this memorandum, to assure that all radiopharmaceuticals be cleared for pharmaceutical quality as is stated in the Director's memorandum, and as approved by the Radiation Committee and Medical Board. However, it should be understood that a tremendous increase in service with the present personnel cannot be offered, since the staff for this

Service, at present, is one person. Other personnel are being trained and can assist for short periods of time but not on a continuing basis.

The major problems consist of suitable space in which to operate. The present space has been satisfactory to a point, but is not suitable in all aspects. A negative pressure and vented hood should be made available in this area to perform several activities. This facility is available in Building 21. However, the unnecessary exposure of the parenteral patient care materials to the environment in Building 21 is not a satisfactory conclusion. Additional space should be provided for those activities which cannot be safely carried on at the present time. Certain administrative functions, such as filing, desk, and library activities must be carried on by this activity. At present it must be maintained in the area where the actual formulation procedures are carried on. This is not safe or desirable for personnel operating in this area over a long period of time. It is not a recommended procedure, and there should be available an office area removed from the formulation area. However, it cannot be removed too far because of the necessity of having past formulation records, computation records for past activities and assays available to the formulation area.

The Chief of the Radiopharmaceutical Service has had several conferences with research personnel of the Department of Radiopharmaceuticals of Abbott Laboratories and Squibb and Company, on the process for producing Iodine 131-Polyvinylpyrrolidione Injection. Both companies have produced pilot batches of this material and it may result in a commercial preparation. This product was first developed by Doctor Gordon in the Heart Institute, and then was produced at a higher level and more consistent purity by the Radiopharmaceutical Service. Both of the pharmaceutical houses mentioned earlier have had great difficulty in preparing this in larger batch lots.

Other activities carried on by the entire Department were as follows: The staff of the entire Department assisted in the closed circuit television demonstration by the Pharmacy Department of various routine techniques, to an all day seminar of The Maryland Association of Hospital Pharmacists on November 24, 1959. This was the first successful use of closed circuit television showing techniques and activities of various services in the Department to a large audience. From honest, unbiased comments, it is felt that this was one of the most successful regional hospital pharmacy seminars ever developed and sponsored in the United States.

The Department has carried on training activities or orientation activities with chief pharmacists of the United Mine Workers during the year. Various visitors from United States and foreign

hospitals have spent from several hours to several days in the Department, learning specific techniques. The Division of Hospitals, Bureau of Medical Services hospital pharmacy interns, are spending a two week period with the Department to obtain training in Central Sterile Supply, Pharmaceutical Development Service and Radiopharmaceutical Service activities; which are generally not available in their own activities. During the fiscal year there are plans to have seven interns take advantage of this activity.

The Department has completed its first full calendar year in offering Pharmacy Officer of The Day Service. It is felt that this has materially assisted in raising the service of the Pharmacy Department by making professional pharmaceutical staff available at all times. The Department no longer has to rely on other personnel to carry on some of its activities. It has been an extra added workload on the professional staff, since these activities are carried on with no compensatory time off. However, this is offset by giving the professional staff a better insight into the problems of the medical and nursing staff, which they cannot obtain during normal working hours, mainly due to the size of the operation.

DEPARTMENT PROBLEMS

One of the most pressing problems is the need for a packaging service space, mentioned earlier, for the Pharmacy Service, the Central Sterile Supply Service and the Pharmaceutical Development Service. It is felt that this area can be fitted into the present Blood Bank space and conversations have been held with the Executive Officer of the Clinical Center to provide this space, when it is vacated. This could also be used, as mentioned earlier, for the highly toxic or unknown toxicity materials being prepared in the Pharmaceutical Development Service, and for the additional ultraviolet hood, aseptic transfer space which is necessary for the Department to provide all needs.

The Department has been hindered by not having an ethylene oxide gas sterilizer available. However, this has been only partly eased as mentioned earlier. The Department still has needs for a large size ethylene oxide gas sterilizer for use with many products which are heat labile, or are injured by heat.

One of the big problems, solved to some extent by the small size ethylene oxide gas sterilizer, has been where sound frequency readings are taken from Cournand Catheters. The autoclaving process completely destroys the property to transmit sound waves, and they must be soaked in sterile liquid material for at least 24 to 48 hours to restore this activity. With the gas sterilization process this can be reduced to approximately a two to four hour soaking period. There are numerous other plastics which should be more suitably

packaged for Clinical Center use. In addition, such items as rubber goods would have their life extended a great deal. As a matter of interest, the operation of a 36" x 48" x 60" gas and steam sterilizer would not be any more costly than the smaller unit, which has just arrived in the Department from the Sanitary Engineering Branch. The reason is that large cylinders of gas would be used.

One other problem is retention of trained personnel in the Department. It commonly happens that once personnel are trained and can function properly, they move on to other positions or leave the Public Health Service. An opening now exists for a Ph.D. as Chief of the Pharmaceutical Development Service in the Department. There has not been a suitable and available Ph.D. applicant in the Pharmacy Corps of the Public Health Service for some six or eight months. This probably is largely due to the fact that once a person obtains a Ph.D., he is eligible for commission in the scientist category, which is an unrestricted category. Why should a Ph.D., with a background in pharmacy apply as a pharmacist, when it is restricted above the Senior Assistant Grade? It is not known how this problem may be overcome, but it will be even a more acute problem starting in January of 1960. This may well affect the department's ability to carry on some of its assigned functions, which have but recently been developed. It may be necessary, through these restrictions, to curtail some of our activities which have been produced in the past, due to inability to obtain properly trained personnel. It is not beyond the realm of possibility to commission a scientist and place him in the Pharmaceutical Development Service. However, it is absolutely essential that he be a registered pharmacist in addition to being a scientist. Furthermore, part of the problem seems to be that a Ph.D. in pharmacy is very rarely interested in coming into the Public Health Service. Salaries paid by industry upon completion of post graduate work are fantastically greater than can be offered to men in the Public Health Service Commissioned Corps. Recently, I requested a pharmacist Ph.D. to enter the Service. However, the salary that could be offered to him under the personnel system after January 1, 1960, would be approximately 50% of his industrial earnings. Under these conditions, I do not know how it will be possible to continue to obtain properly trained personnel for these activities. Prior to reducing our quality, it is felt that it would be better to reduce the quantity of material available.

The Department has continued to cooperate with the Nursing Department in offering an orientation program to new employees, professional and non-professional, by our pharmacists in the various activities of the Department: The metric system, other information concerning supplies, their use, and services available from the Central Sterile Supply Service.

PHARMACY DEPARTMENT STATISTICAL DATA

	1956 (Calendar)	1957 (Calendar)	1958 (Calendar)	1958 (Fiscal)	1959 (Fiscal)
Total number of units issued	2,024,793	2,457,422	3,066,939	2,872,022	3,110,519
Total number of requisitions processed	29,297	33,938	33,232	33,988	33,234
PHARMACY SERVICE					
Prescriptions	11,493	16,034	20,436	17,453	23,775
Other Items	172,687	197,291	208,313	206,440	218,484
Total	184,180	213,325	228,749	223,893	242,259
Requisitions Processed	10,818	12,428	12,547	12,400	12,408
CENTRAL STERILE SUPPLY SERVICE					
Units issued	1,836,665	2,228,207	2,823,340	2,638,179	2,848,488
Requisitions processed	18,164	20,300	19,880	20,489	19,907
Units processed in cooperation with Lab. of Infectious Diseases of NIAID	17,517	38,500	57,725	49,758	69,121
PHARMACEUTICAL DEVELOPMENT SERVICE					
Units Developed and Issued	3,948	15,890	14,081	9,366	18,587
Requisitions processed	315	1,210	678	992	784
Investigational Drugs Registered	- - -	- - -	- - -	- - -	488
RADIOPHARMACEUTICALS					
Units prepared and issued			769	584	1,185
Requisitions processed			127	107	135

PUBLICATIONS, SPEECHES GIVEN, OR SIGNIFICANT ACCOMPLISHMENTS BY PHARMACY DEPARTMENT PERSONNEL INCLUDE

Milton W. Skolaut

Co-author of Hospital Pharmacy Chapter in Husa's Pharmaceutical Dispensing, Fifth Edition, 1959, Mack Publishing Company, with William H. Briner.

Speech at the American Association for the Advancement of Science, Pharmacy Section, December 1959. "Administrative Determinations and Policies Relative to the Use of Radioactive Medications and Investigational Drugs in Hospitals". Co-authored with Dr. Robert M. Farrier.

John A. Scigliano, Ph.D.

Vice-President of Maryland Association of Hospital Pharmacists, 1959.

Lecture delivered to National Institute of Dental Research, stomatitis study group on "Some Considerations in the Development of New Products."

William H. Briner

Co-author of Hospital Pharmacy Chapter in Husa's Pharmaceutical Dispensing, Fifth Edition, 1959, Mack Publishing Company, with Milton W. Skolaut.

Chairman, Committee on Radiopharmaceuticals of The American Society of Hospital Pharmacists, 1959-1960.

Speech, April 21, 1959 to the Senior Administrative Staff Meeting, Nursing Department, Clinical Center, "Radioisotopes, Their Biological Effects and Medical Uses."

Speech, September 28, 1959, at the general staff meeting of the Nutrition Department, Clinical Center, "Radioactivity, Radiopharmaceuticals, A Survey."

Speech, November 18, 1959 at the Scientific Meeting of The American Pharmaceutical Association Student Branch, Temple University School of Pharmacy, Philadelphia, Pennsylvania, "The Public ;Health Service - Mission and Scope."

Speech, November 24, 1959 at the Seminar of Hospital Pharmacy, sponsored by The Maryland Association of Hospital Pharmacists at the Clinical Center, entitled "Nuclear Medicine Has Come of Age."

Samuel Merrill

Training at the Robert A. Taft Engineering Center, Cincinnati, Ohio, "Basic Radiological Health", December 1959.

Walter J. Ludwig

Training at the Robert A. Taft Engineering Center, Cincinnati, Ohio, "Basic Radiological Health", December 1959.

Ray D. Crossley, III

Training at the Robert A. Taft Engineering Center, Cincinnati, Ohio, "Basic Radiological Health", December 1959.

Calendar Year 1959

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

MEDICAL RECORD DEPARTMENT

CC-46
Serial No.

OBJECTIVES:

The Medical Record Department is delegated the major responsibility of insuring the completeness, accuracy, safekeeping and availability of medical records by maintaining the following internal controls: (1) Continual appraisal of the medical records of patients registered at the Clinical Center, in order to maintain the standards established by the Clinical Center and accrediting agencies; (2) Maintenance of required indexes; namely, the analysis of discharged patients and the diseases and operations index (based on Standard Nomenclature of Diseases and Operations), with a view toward providing adequate basic data to physicians participating in medical research; (3) Maintenance, compilation and distribution of statistical data, concerning patient care, for utilization by administrative, clinical and research directors; (4) Maintenance of a systematic arrangement of medical records to insure their immediate accessibility for purposes of patient care service and medical research; (5) Maintenance of a system whereby the quantity and quality of dictated medical record reports are controlled; (6) Maintenance of an adequate control system with regard to the release of medical reports, medical information and medical records to authorized personnel; (7) Maintenance of a perpetual patient's index (Soundex) and patient registry.

PROGRESS MADE:

During the year 1959 there were 3386 medical records received for qualitative and quantitative analyses, posting to discharge analysis, coding and indexing. Also, during the year, 23 research projects, using groups of medical records, were accomplished, and 48 special statistical studies were completed.

To execute basic controls properly, standard operative procedures were supported and new ones developed as follows:

Education and Training:- From March 17, 1959 to May 26, 1959, the Medical Record Department conducted a course in Medical Terminology for medical transcribers and other Medical Record Department personnel.

Medical Record Department

There were two sessions of one hour each held bi-weekly. In addition to classroom work, lectures were presented by physicians and the seven Institutes with a view toward orientation of the class with the anatomy and terminology of their specialties. Twenty-seven employees started the course and twenty-six completed it successfully.

A procedure of orientation was established with a view toward facilitation of modern record keeping by new physicians arriving at the Clinical Center to participate in patient care. This consisted of brochure presentation, question and answer session and lecture tour of the Medical Record Department, all of which, combined, offered clearly defined orientation on the objectives of the research program and recommended modus operandi concerning medical records.

An orientation format was developed for Nursing Department for purposes of education concerning medical records. Also, orientation lecture tours of the Medical Record Department were given to unit clerks, social service workers, United States Public Health Service graduate medical record librarian students and other visitors to the Clinical Center.

Personnel:- Ten positions in the Files Unit and one position in Research and Statistics Section were audited by a Position Classifier.

Procedures Involving Institutes:- New procedures were established with National Cancer Institute and National Institute of Dental Research, with a view toward facilitating the processing of medical reports and medical records more conveniently and promptly by physicians.

A new procedure was established with National Institute of Mental Health regarding registration and medical record requirements for patients admitted for psychological testing.

Procedures Involving Medical Record Department:- A new form "Request for Medical Information" was developed to facilitate the acquisition, processing and furnishing of film, slides, abstracts, and other media from outside agencies to provide better service to physicians participating in patient care.

Due to improved conditions, the Transcribing Unit has maintained its work load and completed a backlog of 500 disks (medical reports) on hand December 30, 1958; at the present time the work is in current status.

A tentative schedule has been established to provide Sunday tour of duty by Files Unit personnel.

Medical Record Department

An "Incomplete Physicians' File" was established in Research and Statistics, listing all physicians participating in patient care and their medical record deficiencies. Part of this procedure is furnishing the physician a list of these deficiencies periodically.

MAJOR PROBLEMS:

Control of medical records during transfer of patients to other areas of observation for purposes of research study and treatment remains unsatisfactory.

Backlog of coding and indexing of medical records (completed through December 31, 1956).

Incomplete medical records (approximately 679 as of December 31, 1959).

Unsuccessful recruitment of one qualified medical record librarian.

A control system for procuring outstanding tissue reports.

PROPOSED FUTURE OBJECTIVES:

Conversion to a unified pre-printed master system for all medical reports with a view toward eliminating bulk, and allowing for a compact narrative report providing continuity of content. This system offers a real service in meeting the high level of complexity involving adequate documented support to physicians engaged in patient care research studies.

Participation by all registered medical record librarians in coding medical records in conformity with the Standard Nomenclature of Diseases and Operations. An "all out" drive for 100% completion of existing backlog of coding is planned.

Preparation of general and specific standards for all positions in the Medical Record Department, and completion of desk audits by Personnel is scheduled.

Conversion of three Patient Registers into one Permanent Patient Registry is in process.

It is hoped to continue the orientation format established in 1959, particularly for new physicians arriving to participate in patient care. These orientation procedures offer clearly defined policies of the research program, and recommended procedures concerning medical

records at the Clinical Center where standards include considerably more than those which suffice for average systems of record keeping established primarily for purposes of diagnosis and treatment. It has been demonstrated that new physicians need this training by the outstanding quality of medical records reviewed in 1959.

It is planned to expand this format in 1960 to include instructions in the use of the Standard Nomenclature of Diseases and Operations, for purposes of coding.

oOo

Medical Record Department

STATISTICS - Calendar Year 1959

I. Registration

A. Patients			5272
Inpatient		3452	
First Registration	1517		
Readmission	1935		
Outpatient (First Registration)		1732	
Walk-In		67	
Emergency		21	
B. Carded for Record Only			3
C. Employee Registration			1770

II. Patient Care Statistics, Calendar Year

Total Number of Admissions	3452
Total Number of Discharges (includes deaths)	3485
Total Patient Days	140,296
Available Bed Days	188,340
Daily Average Number of Patients	384
Average Length of Stay	41
Percentage of Bed Occupancy	74
Total Number of Deaths	255
Total Number of Autopsies	239

Medical Record Department

III. Discharged Inpatients by Institute and Geographic Origin, Calendar Year 1959

REGIONS	NCI	NIAID	NIAMD	NHI	NINDB	NIMH	NIDR	Total Patients
New England	3	10	4	18	21	-	-	56
Middle Atlantic	85	26	61	191	74	18	3	458
East North Central	41	6	27	58	40	15	0	187
West North Central	8	2	3	10	7	11	-	41
South Atlantic	671	321	264	666	451	44	38	2455
East South Central	33	8	23	70	34	-	-	168
West South Central	21	2	3	19	13	1	-	59
Mountain	16	1	1	12	6	1	-	37
Pacific	9	2	4	14	10	2	-	41
U. S. Territories	1	1	-	5	-	-	-	7
Other Non U. S.	8	4	-	7	11	2	-	32
TOTAL PATIENTS	896	383	390	1070	667	94	41	3541
*Washington, D. C.	159	79	42	140	124	8	8	560
*Maryland	207	121	98	158	132	19	25	760
*Virginia	143	118	13	200	63	58	3	598
TOTALS	509	318	153	498	319	85	36	1918

* Said totals are included in reported figures under South Atlantic Region

Medical Record Department

Addendum to Annual Report, 1959 - Discharged Inpatients by Institute and Geographic Origin

A. Grand Total: Discharged Inpatients by Institute and Geographic Origin

CALENDAR YEAR:	1956	1957	1958	1959
	2369	2865	3275	3485

B. Total, Discharged Inpatients by Geographic Area:

Geographic Area	Patients Discharged Calendar Year				Percentage Calendar Year			
	1956	1957	1958	1959	1956	1957	1958	1959
D. C.	551	610	602	560	23	21	18	16
Maryland	589	799	810	760	25	28	25	22
Virginia	377	478	442	598	16	17	13	17
Total	1517	1887	1854	1918	64	66	57	55

Calendar Year 1959

PUBLIC HEALTH SERVICE - NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

NURSING DEPARTMENT

CC-51
Serial No.

A. Program Goals

The goals of the Nursing Department are the same as they have been in the past: (a) provision of high quality nursing care to patients; (b) providing assistance to the clinical investigative staffs; (c) orientation, training and supervision of personnel so they may become more effective in carrying out (a) and (b); and nursing research geared toward improving nursing practices and services to patients.

Each year it has become increasingly difficult to carry out our responsibilities to patients, nursing and medical staffs because of the many and diverse demands for services. Some of our problems will be discussed briefly in another section of this report.

B. Developments and Trends

1. The number of hours of continuous nursing care to patients has shown an overall increase of about 20% during the past year. During calendar year 1958, 50,845¼ hours of continuous nursing care were given. During fiscal year 1959, 59,635 3/4 hours were given. The greatest increases in hours were on the Psychiatric (48.7%), Cancer (40%), Heart (19%), and Allergy and Infectious Diseases (44%) Nursing Services. Fewer hours of continuous nursing care were utilized on the Arthritis and Metabolic (57% less) and Neurology (28% less) Nursing Services.

2. Our experience during the past six and one-half years seems to indicate that we will continue to have difficulty in recruiting and retaining the number of professional nurses that we feel is needed to provide adequately the services requested. Additional part-time nurses have been employed this past year; even so, the number of overtime hours have increased from 5443 (in 1958) to 5965 3/4 during the fiscal year. Also, in order to help meet our needs, we have increased the number of practical nurse positions from 87 to 109.

3. As indicated last year, the number of requests for information and consultation has increased. It has not been possible to respond to all requests and we have been most selective. We have had a number of nurses who have spent from three days to four weeks with us, at their own expense; observing nursing care, attending staff conferences and the like. Several of these nurses have come from abroad. We enjoy sharing with them what we have and what we do, even though at times this becomes something of a hardship on the members of the senior nursing staff.

4. There has been a slight increase in the number of foreign nurse visitors during the past year, and it appears that this activity will continue.

C. Progress Made

We have not been able to do much more than meet the daily demands for services during the past year. This in itself has been quite an accomplishment, and could not have been done without the wholehearted cooperation of all our staff members.

The criteria for promotion have been worked out and promotion rosters developed for two levels of professional nurses-- (1) Staff Nurse GS-6 to Staff Nurse GS-7 and (2) Staff Nurse GS-7 to Head Nurse GS-9.

D. Major Problems

Some of the problems reported in previous years remain unresolved. Requests for nursing services are constantly increasing and it is still difficult to meet them with the staff available to us.

The number of nursing assistants, both men and women, has been increased during the past year. With the increase has been the need to provide additional hours of instruction and supervision in anticipation of further utilization of this group in patient care.

There appears to be a trend toward planning projects around children without consideration of the space and facilities needed. The number of children in the Clinical Center at any one time varies, and there has not been sufficient space for play and recreation for the number of children admitted.

A problem which is becoming more acute is that of space for additional equipment--particularly large or bulky items. During the past year several examining rooms have become overcrowded with equipment for which it was claimed no other space could be found. It is noted, too, that periodically another refrigerator is added to the dumb-waiter lobbies. With each addition of new equipment, existing space for movement of personnel engaged in daily activities with patients has been decreased.

The need for review of the policy regarding the care of patients who develop psychoses, although not acute, is very real. During the past year several patients have demonstrated the need for security measures which are not generally available. When these circumstances arise there is a hazard to patient and personnel, and the time required in constant attendance is considerable.

The readjustment of facilities in the Admissions and Followup area has been accomplished and the recent addition of two examining rooms in the west wing was very welcome. The major problems in this department are associated with the Eye Clinic. Because the receptionist in the Eye Clinic does not come under the same administrative jurisdiction as other receptionists in the outpatient area, there are problems--particularly in communication and coordination of services. Unfortunately, it has not been possible to solve these difficulties thus far.

During the past year the problem of maternity care for patients in advanced stages of pregnancy has risen again. This problem is not new, and with each admission of this type of patient there arises the question of procedure. It would be helpful if some general guidelines could be established, and thus eliminate the time involved in planning on an individual basis on the various services.

The number of employees of the National Institutes of Health seen after hours, on weekends and on holidays has increased by approximately 40%. This problem was mentioned in last year's report and as the number of personnel increases, the problem becomes more burdensome. At the present time the supervisory personnel in the Central Nursing Office are responsible for assisting the Officer of the Day with sick employees after hours. On weekends and holidays a nurse from the staff of the Admissions and Followup Nursing Service is on duty in the Emergency Room to assist with followup patients who are scheduled for visits. In

addition, this nurse has been assisting with employees reporting illnesses. According to the recorded visits, the number of sick employees seen in the Admissions and Followup Department is four times that of followup patients over the past eleven months. Serious consideration should be given to staffing the Employees Health Service seven days each week (including holidays) instead of the present five days.

E. Accomplishments

1. Toward Goals. Every effort has been expended toward carrying out the objectives of our Department. Even with shortages of personnel and an overall average of 30 working days leave (annual, sick and other) per employee, the patients have received a high quality of nursing care. Effective planning, supervision and training of personnel have been the contributing factors.

2. Publications, Honors and Awards. There have been no articles submitted for publication this calendar year. Two are now in preparation for publication.

The Chief, Nursing Department, presented a paper at the Fiftieth Anniversary Celebration of the Vanderbilt University School of Nursing.

Miss Agnes Middleton, Chief, Psychiatric Nursing Service, received an award from the Commissioners of the District of Columbia for her participation on the Public Health Council.

Serial No. CC-51
1. Clinical Center
2. Nursing Department

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

A Study of the Weighing of Patients by Nursing Personnel at the Clinical Center, NIH

Principal Investigator:

Jane Wilcox
Special Assistant for Nursing Research
Nursing Department
Clinical Center

Other Investigators:

None

Cooperating Units:

None

Man Years:

Total	11/12 man year
Professional:	5/12 man year
Other:	1/2 man year

Project Description:

Purpose:

To improve the quality of nursing participation in clinical research at the Clinical Center by studying the accuracy and precision of weighing patients by nursing personnel.

Objectives:

(1) To evaluate the equipment and procedures used, and the performance of nursing staff in terms of accuracy and precision achieved.

(2) To derive an estimate of the accuracy and precision achieved in the weighing of patients in order to relate this to the level of accuracy and precision needed in various clinical investigations.

(3) To explore methods of investigation that may be useful in further studies.

Methods:

(1) A rigorous inspection and testing of a representative sample of scales currently used for weighing patients in accordance with principles and methods established by the National Bureau of Standards.

(2) A series of experiments in which various categories of the nursing staff performed weighings on the several types of scales used.

Major Findings:

(1) Under the existing system of scale maintenance and inspection, many of the scales are in such condition that they will not weigh reliably at the level which was intended by the manufacturer.

(2) Certain scales show a high order of reliability under test conditions, but when used by the nursing staff, the results are considerably less accurate and precise because of such factors as complexity of operation, difficulty in reading and the like.

(3) Weighing of patients in metabolic balance studies is accomplished with a high order of accuracy and precision.

(4) The weighing of patients is done in an overcrowded physical environment. This interferes both with staff performance and with proper safeguarding of scales.

(5) There is no evidence of measurable differences in accuracy and precision among various categories (grade levels) of nursing personnel in weighing patients.

Significance of Project:

(1) The findings will enable the Nursing Department to plan more effectively with clinical investigators for achieving the order of reliability needed in the weighing of patients in clinical research projects.

(2) Certain findings and recommendations will enable the Nursing Department to improve those aspects of patient weighing for which they are responsible.

(3) Through the medium of Nursing Research, which has asked to publish a report of the study, the methodology and general conclusions can be shared with other nurses interested in improving the quality of nursing practice in this area.

(4) The experience of this study will facilitate further study of nursing practices which are important to the conduct of clinical research.

Part B.

Not included.

Serial No. CC 5
1. Clinical Center
2. Nursing Department

PHS-NIH
Individual Project Report
Calendar Year 1959

Part A.

Project Title:

A Study of Variability Among Nursing Personnel of the Clinical Center, NIH, in the Measurement of Blood Pressure

Principal Investigator:

Jane Wilcox
Special Assistant for Nursing Research
Nursing Department
Clinical Center

Other Investigators:

None

Cooperating Units:

Johns Hopkins University
School of Hygiene and Public Health
Heart Disease Control Program, B.S.S., PHS

Man Years:

Total:	5/6 man year
Professional:	5/12 man year
Other:	5/12 man year

Project Description:

Purpose:

To improve the quality of nursing participation in clinical research at the Clinical Center, by studying the contribution of observer factors to the error of measurement in blood pressure readings performed by professional and practical nurses at the Clinical Center.

Objectives:

(1) To determine the order of magnitude of the variability among professional nurses and practical nurses, so clinical investigators may take it into account in the conduct and planning of their research.

(2) To compare the performance of professional versus practical nurses in measuring blood pressure as a basis for planning of assignments of duties.

(3) To examine the effects on observer variability of certain characteristics such as age, visual or hearing defects, experience and training as a basis for planning assignment of duties and in-service training.

(4) To examine the effects on observer variability of certain elements of the procedure for measuring blood pressure as a basis for planning in-service training and supervision of staff.

Methods:

(1) A series of experiments with observers using a double stethoscope (two sets of ear pieces) and designed to obtain estimates of inter-observer variability independent of the variability of the blood pressure level itself.

(2) Experiments to study the effects of the rate of the lowering of the mercury in the sphygmomanometer and the method of application and positioning of the blood pressure cuff.

(3) The use of a sound motion picture showing a mercury column and giving the sound normally heard through a stethoscope, so that readers can make measurements of blood pressure of a series of subjects: Control Program, BSS and the Johns Hopkins School of Hygiene and Public Health.

Major Findings:

Data Collection has been completed and data is being analyzed and interpreted. Final report will be available early in 1960.

Preliminary results suggest that findings will have practical application to nursing practice at the Clinical Center, and will provide clinical investigators with useful estimates of the error of measurement introduced by nurse observers.

Part B.

Not included.

Calendar Year [illegible]

PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

SUMMARY ANNUAL REPORT OF PROGRAM ACTIVITIES
CLINICAL CENTER

NUTRITION DEPARTMENT

[illegible]
Serial No.

I. CONTRIBUTIONS TO THE CLINICAL RESEARCH PROGRAM

In making its contribution to the Clinical Research Program, the Nutrition Department provides research investigators with precise, detailed, and complete data on patient nutrient intake to assist the investigators in: Interpreting the mode of action of new drugs; Seeking additional knowledge of diseases for the purpose of developing new medications; Evaluating the course of a disease or the status of a patient; Diagnosis.

To provide this data, the Nutrition Department assumes varied and unique responsibilities:

One of these is that of controlling a single nutrient intake in varying amounts for an individual patient. For example the NHI uses controlled sodium intakes and controlled tryptophane intakes extensively in evaluating new drugs for treatment and control of hypertension. The NIAMD uses purine free diets, controlled protein intakes, and maintenance calories in its studies of uric acid metabolism.

Another responsibility is that of controlling more than one nutrient intake for purposes of therapy or diagnosis for an individual patient. These diets have been used by the NIAMD in calcium studies and by the NIAID. Controlling nutrient and/or calorie intakes for individual patients to be iso-nutrient or iso-caloric, or both, daily, is a dietary control under which a diet of very nearly the same composition is calculated daily and food refused is replaced so that the nutrient and calorie intakes of the patient stay essentially the same. NHI orders these diets for long term studies on patients and normal controls to compare the cholesterol levels during ingestion of different types of fats, to test and evaluate effects of various types of fats on blood lipids in fat studies of atherosclerosis. These are also used by NINDB in its study of periodic familial paralysis.

Diets which control group intakes qualitatively--as control groups and therapy groups-- are ordered by NIMH in its study of the biochemical aspects and influence of histidine in the metabolism of schizophrenics.

Diets involving numerous controls are provided for short term studies or tests, such as the "constant regular diet" used by NCI in its "2 Deoxy-D-Glucose" study, under which the patient must be served the same specific foods at the same time each day, and those used by the NIAMD and NIAID for diagnostic purposes.

Semi-synthetic liquid diets prepared and administered for the purpose of supplying protein, fat, carbohydrate, and calories in specific amounts have been ordered by NIAID and NIDR to investigate possible allergic reactions to foods in aphthous stomatitis patients, and by NCI to determine the usefulness of a chemically defined water soluble diet composed of vitamins, minerals, amino acids, carbohydrate, and fat in future studies requiring purified diets.

The Department measures the daily nutrient intakes of individual patients. This type of diet is used by NCI, Pediatrics, in its study of nucleic acid metabolites in patients with acute leukemia.

The four Metabolic Kitchen units, because of facilities, food items, and staff needed to meet the requirements, provide diets for a variety of balance studies. Typical of these diets are the pantothenic acid deficient synthetic diets provided by the 3-B Metabolic Kitchen for NCI to study the effect of Omega-Methyl pantothenate (the analogue of pantothenic acid); diets so calculated that the acid residue of foods balances the basic residue of foods, provided by the 7th Metabolic Kitchen to NHI; and those furnished by the 9th Metabolic Kitchen for NIAMD to study the effect of various levels of calcium intake with and without Vitamin D.

The foregoing describes only a few of the Nutrition Department's responsibilities, but demonstrates the exacting details of its work, the extreme importance of close supervision of non-professional personnel, and its need for professional know-how. The complexity and variation of the Department's daily accomplishments is brought out by the fact that while the work described above usually involves only one patient, the Department daily renders this same exacting and detailed service to many patients simultaneously.

II. MAJOR PROBLEMS ENCOUNTERED

Workload fluctuation is probably the greatest problem in the Nutrition Department because of the inflexible staffing pattern. Dietary service to patients subject to medical research requires continuous expert professional supervision so that to provide research services, a trained staff, professional and non-professional, must be maintained

regardless of workload. The number of diets required fluctuates erratically. For example, in March 1959, 2100 Categories III and IV diet meals were served to patients of NIMH. Just one month later, only a few more than 600 meals were served--a decrease of 1500 meals. (By definition, Categories III and IV diets reflect the research need).

Rising costs of personnel and the fluctuating raw food market cause an unceasing struggle in the Department to keep within the allotted budget. Personnel costs increased greatly this year as a result of the pay raise granted wage board personnel (80% of the Nutrition Department staff) on January 11, 1959. As a direct result, the patient ration cost rose; cafeteria prices had to be increased; food items showing marked price increases were eliminated from the cafeteria menu; and the size of portions was reduced. The very lowest level of expenditure for raw food possible under a program maintaining quality food standards was attained in the cafeteria operation. Raw food costs in general have shown a tendency to increase and the market is unpredictable.

Other problems are those of over-crowded facilities in the Main Kitchen and cafeteria; insufficient space in Diet offices on several floors; the need for space for conferences and training classes; the need for air-conditioning in the Main Kitchen; the presence of insects in the Main Kitchen (this has been greatly alleviated through mutual cooperation of this Department and Sanitary Engineering); shortages in cash boxes of two Department cashiers who were subsequently separated from the service; the lack of an overall preventive maintenance program for equipment; impractical design of hot food carts used in the patient area--designed for large quantities of food and fail to keep hot the small quantities served to patients on special diets; advancing inefficiency and increasing difficulty in repairing dishwashing machines in floor kitchens. Also, the 13th Floor Kitchen, in addition to its regular workload, served the patients of the 3-B Metabolic Kitchen during the three months it was closed.

III. ACCOMPLISHMENTS, CHANGES AND IMPROVEMENTS

In the interest of brevity, only the high lights of the year's accomplishments, changes and improvements are reported here.

For the first time, the Department was able to report some of its nutrition observations to the dietetic profession. A report of our study, "The Factors Affecting Calorie Intakes of Undernourished Patients," was submitted to the Journal of the American Dietetic Association.

The first draft of the Diet Manual was completed and a final copy is in preparation for presentation to the Director of the Clinical Center.

During the period December 1, 1958, through November 30, 1959, the Nutrition Department provided 22,240 recorded daily dietary intakes (an average of more than 60 a day), 402,576 patients' meals, and weighed food on 200,160 individual trays. In addition, 128 diet aliquots (384 meals) were weighed and provided for laboratory analysis, 11,425 patient days of fluid intakes were measured, and 263 individual patients were given discharge or follow-up diet instructions, each requiring a complete nutrition history.

Twelve floor kitchens, a formula room, the Main Kitchen, and the 9th floor Metabolic Kitchen (servicing NIAMD and NCI) were operated the full twelve months of the year. The kitchen in the residential treatment facility, T-4, closed on June 23, 1959. The 7th Metabolic Kitchen (servicing NHI) was open 11 months and the 3-B Metabolic Kitchen (servicing NCI), 9 months. The fourth Metabolic Kitchen (12th floor) was opened on October 26, 1959, providing a second such unit to service NCI.

In addition to the patient area food service, the Nutrition Department operated a public cafeteria serving National Institutes of Health Personnel and visitors.

The Department reviewed and improved procedures and established new ones where needed. Arrangements were made with the Admissions and Follow-up Department for out-patients to receive diet instructions, on a consultative basis, through that department; the procedure for writing diets for NIMH was revised--instead of serving the same menu for all five mental health patient groups as before, the food preferences of each group are considered and a specific menu written for each group, providing patients with a better and more individualized food choice; a radiation isolation procedure for handling patients' trays was established for use by Nutrition personnel and nursing in the radiation service of NCI; a cafeteria counter supervisor was added to the staff to extend supervisory coverage from five to seven days a week; two positions were added to the Main Kitchen area staff to carry out newly established special cleaning procedures, including steam cleaning of all areas in the Main Kitchen and cafeteria counter; the Department piloted a procedure for assigning lockers and handling keys for its employees; cashiers are authorized to obtain change from the Clinical Center Agent Cashier when the bank is closed and each cashier is provided with a separate cash box and money bank; the Chief, Patient Dietetic Service Branch, effected a program for the weekly inspection of Floor Kitchens with written reports and follow-up staff meetings; the detailed procedures for the Formula Room were completely revised; the food groups under which all food items used by the Department are classified for accounting purposes were revised and reduced from 15 to 13; the professional staff completed a "Food Portions Manual," a manual for training Food Production Service Dietitians, and a compilation, in book form, of Patient Dietetic Service procedures.

In the field of Personnel Management, the Department completed a comprehensive review of its staffing structure; developed criteria for successful job performance for each of its non-professional positions and established a promotion policy for these positions; reviewed their job descriptions with all clerical and non-professional personnel and re-wrote the descriptions where necessary; scheduled a 15 minute break, once a day, for non-professional staff; reorganized the responsibilities of the Food Production Service professional staff; arranged for the Fire Marshall, NIH, to review fire prevention and control precautions with Main Kitchen supervisors and dietitians.

Under the Nutrition Department training program, non-professional staff training classes covered the topics, (1) "The Organizational Structure," (2) "Means of Communicating in Our Work," (3) "The Importance of Listening--to an Efficient Operation," and (4) "Food Cost Control Through Accurate Ordering, Inventory, Food Preparation and Food Service."

The installation, throughout the year, of many items of new equipment in the Patient area, Main Kitchen, and Cafeteria, improved efficiency and manpower utilization. Especially noteworthy was the installation in all floor kitchens of rinse injector units and "sterile-sil" baskets which brought under control a high bacterial count on silverware in the Patient area. Facilities in the Main Kitchen and Cafeteria area were improved through painting, installation of a new drainage system, remodeling and repairs, some of which were part of the insect extermination project carried out cooperatively between the Nutrition Department and Sanitary Engineering. Installation of clocks in the women's locker room and the salad unit of the Main Kitchen area and an intercom system between the cafeteria, bakeshop, salad unit and cooks' area proved especially helpful.

An additional office was allotted to the Patient Dietetic Service, 13th floor, relieving a crowded situation there.

IV. PROPOSED FUTURE OBJECTIVES

In addition to carrying out its basic responsibility of rendering quality food service throughout the Clinical Center, the Nutrition Department's plans for the future include:

Continued emphasis on development of more efficient operating procedures in practical dietary application to research programs and reporting same.

Continuation, for further investigation and evaluation of material for the diet manual and possible publication, of a study of the actual calorie intake of an average Clinical Center patient on a regular diet.

Printing of the revision of the manual of current diet procedures.

Expanded activity in nutritional research studies and their publication so that our administrative practices and experiences may be shared with others in the field of research.

Continuation of the project of abstracting current nutrition literature and distributing it as a bulletin to staff members expressing specific needs.

A comprehensive review of the Department's staffing structure in light of rising costs and changing programs.

Continuation of in-service training for professional and clerical staff.

Development with the Board of Civil Service Examiners of a new examination and set of standards for Nutrition Department non-professional staff.

Seeking better methods of selecting food service workers and supervisors in cooperation with the Civil Service Commission Test Development Section.

A study of the relationship between attendance records and reasons for illness of food service workers.

Re-evaluation, after six months' experience with it, of the Nutrition Department promotion policy.

Evaluation of the need for nutritional services in the Follow-up Unit to determine whether the professional position in that area should be activated.

Planning for the remodeling and expansion of the Cafeteria to provide additional seating for 250 and a third serving line. The third serving line to be a short "soup and sandwich line".

Library
National Institutes of
Bethesda Maryland

LIBRAR
NIH
Amazing Rese
Amazing Help
http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-11
301-496-1080

DATE DUE

GAYLORD
PRINTED IN U.S.A.

NIH LIBRARY
4 0131 7228

BOUND BY
HM

NIH LIBRARY

3 1496 00198 0609